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(54) Title: DETECTION AND MODULATION OF THE IAPS AND NAIP FOR THE DIAGNOSIS AND TREATMENT OF PROLIFERATIVE DISEASE

(57) Abstract

Disclosed are diagnostic and prognostic methods and kits for the detection and treatment of proliferative diseases such as cancer (e.g., ovarian cancer, breast cancer, and lymphoma). Also disclosed are therapeutics for treating proliferative diseases (and methods for identifying such therapeutics) that utilize IAP and NAIP antisense nucleic acid molecules, antibodies which specifically bind IAP and NAIP polypeptides, and compounds that reduce the biological activities of IAP and NAIP polypeptides.

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# DETECTION AND MODULATION OF THE IAPs AND NAIP FOR THE DIAGNOSIS AND TREATMENT OF PROLIFERATIVE DISEASE

## Background of the Invention

The invention relates to the diagnosis and treatment of proliferative disease, in particular, cancer.

One mechanism by which cells die is referred to as apoptosis, or programmed cell death. Apoptosis often occurs as a normal part of the development and maintenance of healthy tissues, and is now known to play a critical role in embryonic development. The failure of a normal apoptotic response has been implicated in the development of cancer; autoimmune disorders, such as lupus erythematosis and multiple sclerosis; and in viral infections, including those associated with herpes virus, poxvirus, and adenovirus.

Compared to the numerous growth promoting genes identified to date (>100) relatively few genes have been isolated that regulate apoptosis. Baculoviruses encode proteins termed inhibitors of apoptosis proteins (IAPs) which inhibit the apoptosis that would otherwise occur when insect cells are infected by the baculovirus. The baculovirus IAP genes include sequences encoding a ring zinc finger-like motif (RZF), which is presumed to be directly involved in DNA binding, and two N-terminal domains that consist of a 70 amino acid repeat motif termed a BIR domain (Baculovirus IAP Repeat). Mammalian IAP family members, and a related anti-apoptotic polypeptide, NAIP, have recently been identified.

Both normal cell types and cancer cell types display a wide range of susceptibility to apoptotic triggers. Many normal cell types undergo temporary growth arrest in response to a sub-lethal dose of radiation or cytotoxic chemical, while cancer cells in the vicinity undergo apoptosis. This provides the crucial treatment "window" of appropriate toxicity that allows successful anti-cancer therapy. It is therefore not surprising that resistance of tumor cells to apoptosis is emerging as a major category of cancer treatment failure. Finding compounds which overcome or prevent this resistance would greatly improve cancer therapies.

## Summary of the Invention

We have discovered that IAP and NAIP overexpression are specifically associated with a wide range of cancer types including ovarian cancer, adenocarcinoma, lymphoma, and

pancreatic cancer. The presence of a fragmented IAP polypeptide in the nucleus, and an overexpression of an IAP polypeptide in the presence of a p53 mutation correlates with a cancer diagnosis, a poor prognosis, and a resistance to numerous chemotherapeutic cancer drugs. In addition, we have found that an therapeutic agent that reduces the biological activity of an IAP polypeptide will induce apoptosis in a cell expressing the polypeptide (*e.g.*, a cell that is proliferating in a proliferative disease). These discoveries provide diagnostic and prognostic methods for the detection and treatment of proliferative diseases, and provide therapeutic compounds useful for the treatment of proliferative diseases, particularly cancer.

In a first aspect, the invention features a method for enhancing apoptosis in a cell from a mammal with a proliferative disease, the method including administering to the cell a compound that inhibits the biological activity of an IAP polypeptide or a NAIP polypeptide, the compound being administered to the cell in an amount sufficient to enhance apoptosis in the cell. In one embodiment of this aspect of the invention, the cell is proliferating in the proliferative disease. In another embodiment, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of polypeptide present in the cell); the level of expression of an mRNA molecule encoding the polypeptide; or an apoptosis-inhibiting activity.

In various embodiments of the first aspect of the invention, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiment, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2. In other preferred embodiments, the mammal is a human or a mouse, and the proliferative disease is cancer, for example, a cancer in a tissue selected from the group consisting of ovary, breast, pancreas, lymph node, skin, blood, lung, brain, kidney, liver, nasopharyngeal cavity, thyroid, central nervous system, prostate, colon, rectum, cervix, endometrium, and lung.

In various preferred embodiments of the first aspect of the invention, the compound is a negative regulator of an IAP or an NAIP-dependent anti-apoptotic pathway; a fragment of the IAP polypeptide, the fragment including a ring zinc finger and having no more than two BIR domains; a nucleic acid molecule encoding a ring zinc finger domain of the IAP polypeptide; a compound that prevents cleavage of the IAP polypeptide or the NAIP polypeptide; a purified antibody or a fragment thereof that specifically binds to the IAP polypeptide or the NAIP polypeptide; a ribozyme; or an antisense nucleic acid molecule have

a nucleic acid sequence that is complementary to the coding strand of a nucleic acid sequence encoding the IAP polypeptide or the NAIP polypeptide. Preferably, the cleavage is decreased by at least 20% in the cell; the antibody binds to a BIR domain of the IAP polypeptide or the NAIP polypeptide; the nucleic acid sequence encoding the IAP polypeptide or the NAIP polypeptide has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, or the nucleic acid sequence of NAIP; the antisense nucleic acid molecule decreases the level of the nucleic acid sequence encoding the IAP polypeptide or the NAIP polypeptide by at least 20%, the level being measured in the cytoplasm of the cell; the antisense nucleic acid molecule is encoded by a virus vector; or the antisense nucleic acid molecule is encoded by transgene.

In a second aspect, the invention features a method for detecting a proliferative disease or an increased likelihood of the proliferative disease in a mammal that includes: (a) contacting an IAP or a NAIP nucleic acid molecule that is greater than about 18 nucleotides in length with a preparation of nucleic acid from a cell of the mammal, the cell proliferating in the disease, the cell from a tissue; and (b) measuring the amount of nucleic acid from the cell of the mammal that hybridizes to the molecule, an increase in the amount from the cell of the mammal relative to a control indicating a an increased likelihood of the mammal having or developing a proliferative disease. In one embodiment, the method further includes the steps of: (a) contacting the molecule with a preparation of nucleic acid from the control, wherein the control is a cell from the tissue of a second mammal, the second mammal lacking a proliferative disease; and (b) measuring the amount of nucleic acid from the control, an increase in the amount of the nucleic acid from the cell of the mammal that hybridizes to the molecule relative to the amount of the nucleic acid from the control indicating an increased likelihood of the mammal having or developing a proliferative disease.

In one embodiment of the methods of the second aspect of the invention, the method further includes the steps of: (a) providing a pair of oligonucleotides having sequence identity to or being complementary to a region of the IAP or the NAIP nucleic acid molecule; (b) combining the pair of oligonucleotides with the nucleic acid under conditions suitable for polymerase chain reaction-mediated nucleic acid amplification; and (c) isolating the

amplified nucleic acid or fragment thereof. Preferably, the amplification is carried out using a reverse-transcription polymerase chain reaction (*e.g.*, RACE).

In one embodiment of the second aspect of the invention, the method provides measuring the nucleic acid having a nucleotide sequence that has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, or the nucleic acid sequence of NAIP. In other embodiments, the method provides measuring the nucleic acid having a nucleotide sequence that has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, or NAIP.

In a third aspect, the invention features a method for detecting a proliferative disease or an increased likelihood of developing the disease in a mammal, the method including measuring the level of biological activity of an IAP polypeptide or a NAIP polypeptide in a sample of the mammal, an increase in the level of the IAP polypeptide or the NAIP polypeptide relative to a sample from a control mammal being an indication that the mammal has the disease or increased likelihood of developing the disease. In various embodiments, the sample includes a cell that is proliferating in the disease from the mammal, the cell from a tissue; and the sample from a control mammal is from the tissue, the sample consisting of healthy cells. In another embodiment, the mammal and the control mammal are the same.

In various embodiments of the third aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

In a fourth aspect, the invention features a method for identifying a compound enhances apoptosis in an affected cell that is proliferating in a proliferative disease that includes exposing a cell that overexpresses an IAP polypeptide or a NAIP polypeptide to a candidate compound, a decrease the level of biological activity of the polypeptide indicating the presence of a compound that enhances apoptosis in the affected cell that is proliferating in the proliferative disease.

In a fifth aspect, the invention features a method for identifying a compound that enhances apoptosis in an affected cell that is proliferating in a proliferative disease that includes the steps of: (a) providing a cell including a nucleic acid molecule encoding a IAP polypeptide or a nucleic acid molecule encoding a NAIP polypeptide, the nucleic acid molecule being expressed in the cell; and (b) contacting the cell with a candidate compound and monitoring level of biological activity of the IAP polypeptide or the NAIP polypeptide in the cell, a decrease in the level of biological activity of the IAP polypeptide or the NAIP polypeptide in the cell in response to the candidate compound relative to a cell not contacted with the candidate compound indicating the presence of a compound that enhances apoptosis in the affected cell that is proliferating in the proliferative disease. Preferably, the cell further expresses a p53 polypeptide associated with the proliferative disease.

In various embodiments of the fourth and fifth aspects of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

In a sixth aspect, the invention features a method for determining the prognosis of a mammal diagnosed with a proliferative disease that includes the steps of: (a) isolating a sample from a tissue from the mammal; and (b) determining whether the sample has an increased an level of biological activity of an IAP polypeptide or an NAIP polypeptide relative to a control sample, an increase in the level in the sample being an indication that the mammal has a poor prognosis. In various embodiments of this aspect of the invention, the sample includes a cells that is proliferating in the proliferative disease and the control sample is from the tissue, the control sample consisting of healthy cells; and the sample and the control sample are from the mammal. Preferably, the sample further includes a cell expressing a p53 polypeptide associated with the proliferative disease.

In various embodiments of the sixth aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression

of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2. In a preferred embodiment, the level is assayed by measuring the amount of IAP peptide of less than 64 kDa present in the sample.

In a seventh aspect, the invention features a method for determining the prognosis of a mammal diagnosed with a proliferative disease that includes the steps of: (a) isolating a sample from the mammal, the sample having a nuclear fraction; and (b) measuring the amount of a polypeptide that is recognized by an antibody that specifically binds an IAP polypeptide or an antibody that specifically binds an NAIP polypeptide in the nuclear fraction of the sample relative an amount from a control sample, an increase in the amount from the sample being an indication that the mammal has a poor prognosis. In preferred embodiments of this aspect of the invention, the sample is from a tissue of the mammal, the sample including a cell that is proliferating in the proliferative disease, and the control sample is from the tissue, the control sample consisting of healthy cells. In another embodiment, the sample and the control sample are from the mammal.

In various embodiments of the seventh aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2. In another embodiment, the amount is measured by immunological methods.

In an eighth aspect, the invention features a method for treating a mammal diagnosed as having a proliferative disease that includes the steps of: (a) measuring the amount of an IAP or NAIP polypeptide in a first sample from a tissue from the mammal, the first sample including a cell that is proliferating in the proliferative disease; (b) measuring the amount of the polypeptide in a second sample from the tissue, the second sample consisting of healthy cells; (c) detecting an increase in the amount of the polypeptide in the first sample to the

amount of the polypeptide in the second sample; and (d) treating the mammal with a compound that decreases the biological activity of the polypeptide. Preferably, the first sample and the second sample are from the mammal.

In various embodiments of the eighth aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

In a ninth embodiment, the invention features the use of a compound that decreases the biological activity an IAP polypeptide or a NAIP polypeptide for the manufacture of a medicament for the enhancement of apoptosis.

In various embodiments of the ninth aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

In a tenth aspect, the invention features a kit for diagnosing a mammal for the presence of a proliferative disease or an increased likelihood of developing a proliferative disease, the kit compromising an oligonucleotide that hybridizes to a nucleic acid sequence that encodes an IAP polypeptide or a NAIP polypeptide.

In various embodiments of the tenth aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

In an eleventh aspect, the invention features a transgenic mammal, the mammal having an elevated level of biological activity of an IAP polypeptide or a NAIP polypeptide.

In various embodiments of the eleventh aspect of the invention, the biological activity is the level of expression of the polypeptide (measured, for example, by assaying the amount of the polypeptide present in the cell); wherein the biological activity is the level of expression of an mRNA molecule encoding the polypeptide; or wherein the biological activity is an apoptosis-inhibiting activity. In another embodiment, the polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP. In other embodiments, the polypeptide is NAIP, XIAP, HIAP-1, or HIAP-2.

By "IAP gene" is meant a gene encoding a polypeptide having at least one BIR domain and is capable of modulating (inhibiting or enhancing) apoptosis in a cell or tissue when provided by other intracellular or extracellular delivery methods (see, *e.g.*, the U.S.S.N.s 08/511,485, 08/576,965, and PCT/1B96/01022). In preferred embodiments the IAP gene is a gene having about 50% or greater nucleotide sequence identity to at least one of the IAP amino acid encoding sequences of Figs. 1-6 (SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, and SEQ ID NO: 13) or portions thereof, or has a ring zinc finger domain. Preferably, the region of sequence over which identity is measured is a region encoding at least one BIR domain and a ring zinc finger domain. Mammalian IAP genes include nucleotide sequences isolated from any mammalian source. Preferably, the mammal is a human. The term "IAP gene" is meant to encompass any member of the family of genes that encode inhibitors of apoptosis. An IAP gene may encode a polypeptide that has at least 20%, preferably at least 30%, and most preferably at least 50% amino acid sequence identity with at least one of the conserved regions of one of the IAP members described herein (*i.e.*, either the BIR or ring zinc finger domains from the human or murine XIAP, HIAP-1, or HIAP-2). Representative members of the IAP gene family include, without limitation, the human and murine XIAP, HIAP-1, or HIAP-2 genes.

By "a virus vector" is meant a functional or attenuated virus that is capable of delivering to a virus-infected cell a nucleic acid molecule. Preferably, the virus vector has been genetically engineered according to standard molecular biology techniques to bear a

heterologous nucleic acid molecule. Virus vectors include, without limitation, adenoviruses, retroviruses, baculoviruses, cytomegaloviruses (CMV), and vaccinia viruses.

By "IAP protein" or "IAP polypeptide" is meant a polypeptide, or fragment thereof, encoded by an IAP gene.

By "NAIP gene" and "NAIP polypeptide" is meant the NAIP genes, fragments thereof, and polypeptides encoded by the same described in UK9601108.5 filed January 19, 1996 and PCT Application No. PCT/IB97/00142 (claiming priority from UK9601108.5) filed January 17, 1997.

By "BIR domain" is meant a domain having the amino acid sequence of the consensus sequence: Xaal-Xaal-Xaal-Arg-Leu-Xaal-Thr-Phe-Xaal-Xaal-Trp-Pro-Xaa2-Xaal-Xaal-Xaa2-Xaa2-Xaal-Xaal-Xaal-Xaal-Leu-Ala-Xaal-Ala-Gly-Phe-Tyr-Tyr-Xaal-Gly-Xaal-Xaal-Asp-Xaal-Val-Xaal-Cys-Phe-Xaal-Cys-Xaal-Xaal- Xaal-Xaal-Xaal-Xaal-Trp-Xaal-Xaal-Xaal-Asp-Xaal-Xaal-Xaal- Xaal-Xaal-His-Xaal-Xaal-Xaal-Xaal-Pro-Xaal-Cys-Xaal-Phe-Val, wherein Xaal is any amino acid and Xaa2 is any amino acid or is absent (SEQ ID NO: 2). Preferably, the sequence is substantially identical to one of the BIR domain sequences provided for XIAP, HIAP-1, or HIAP-2 herein.

By "ring zinc finger" or "RZF" is meant a domain having the amino acid sequence of the consensus sequence: Glu-Xaal-Xaal-Xaal-Xaal-Xaal-Xaal-Xaa2-Xaal-Xaal-Xaal-Cys-Lys-Xaa3-Cys-Met-Xaal-Xaal-Xaal-Xaal-Xaal-Xaa3-Xaal-Phe-Xaal-Pro-Cys-Gly-His-Xaal-Xaal-Xaal-Cys-Xaal-Xaal-Cys-Ala- Xaal-Xaal-Xaal-Xaal-Xaal-Cys-Pro-Xaal-Cys, wherein Xaal is any amino acid, Xaa2 is Glu or Asp, and Xaa3 is Val or Ile (SEQ ID NO:1).

Preferably, the sequence is substantially identical to the RZF domains provided herein for the human or murine XIAP, HIAP-1, or HIAP-2.

By "enhancing apoptosis" is meant increasing the number of cells which apoptose in a given cell population. Preferably, the cell population is selected from a group including ovarian cancer cells, breast cancer cells, pancreatic cancer cells, T cells, neuronal cells, fibroblasts, or any other cell line known to proliferate in a laboratory setting. It will be appreciated that the degree of apoptosis enhancement provided by an apoptosis enhancing compound in a given assay will vary, but that one skilled in the art can determine the statistically significant change in the level of apoptosis which identifies a compound which enhances apoptosis otherwise limited by an IAP. Preferably, "enhancing apoptosis" means

that the increase in the number of cells undergoing apoptosis is at least 25%, more preferably the increase is 50%, and most preferably the increase is at least one-fold. Preferably, the sample monitored is a sample of cells which normally undergo insufficient apoptosis (*i.e.*, cancer cells).

By "proliferative disease" is meant a disease which is caused by or results in inappropriately high levels of cell division, inappropriately low levels of apoptosis, or both. For example, cancers such as lymphoma, leukemia, melanoma, ovarian cancer, breast cancer, pancreatic cancer, and lung cancer are all examples of proliferative disease. A neoplasm (*i.e.*, any abnormal proliferation of cells, malignant or benign), is also a proliferative disease of the invention.

By a "cell proliferating in a proliferative disease" is meant a cell whose abnormal proliferation contributes to the disease. Preferably, the cell expresses the antigen PCNA.

By "polypeptide" is meant any chain of more than two amino acids, regardless of post-translational modification such as glycosylation or phosphorylation.

By "IAP or NAIP biological activity" is meant any activity known to be caused *in vivo* or *in vitro* by a NAIP or an IAP polypeptide. Preferred biological activities of IAP and NAIP polypeptides are those described herein, and include, without limitation, a level of expression of the polypeptide that is normal for that cell type, a level of expression of the mRNA that is normal for that cell type, an ability to block apoptosis, and an ability to be cleaved.

By a "compound that decreases the biological activity" is meant a compound that decreases any activity known to be caused *in vivo* or *in vitro* by a NAIP polypeptide or an IAP polypeptide. Preferred compounds include, without limitation, an antisense nucleic acid molecule that is complementary to the coding strand of nucleic acid molecule that encodes an IAP or a NAIP polypeptide; an antibody, such as a neutralizing antibody, that specifically binds to an IAP or a NAIP polypeptide; and a negative regulator of an IAP or a NAIP polypeptide, such as a polypeptide fragment that includes the ring zing finger of an IAP polypeptide, a polypeptide fragment that has no more than two BIR domains, or nucleic acid molecules encoding these polypeptide fragments.

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 50%, preferably 85%, more preferably 90%, and most preferably 95% homology to a

reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 50 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

Sequence identity is typically measured using sequence analysis software with the default parameters specified therein (*e.g.*, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). This software program matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "substantially pure polypeptide" is meant a polypeptide that has been separated from the components that naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the polypeptide is an IAP polypeptide that is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, pure. A substantially pure IAP polypeptide may be obtained, for example, by extraction from a natural source (*e.g.* a fibroblast, neuronal cell, or lymphocyte) by expression of a recombinant nucleic acid encoding an IAP polypeptide, or by chemically synthesizing the protein. Purity can be measured by any appropriate method, *e.g.*, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A protein is substantially free of naturally associated components when it is separated from those contaminants which accompany it in its natural state. Thus, a protein which is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure polypeptides include those derived from eukaryotic organisms but synthesized in *E. coli* or other prokaryotes.

By "substantially pure DNA" is meant DNA that is free of the genes which, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (*e.g.*, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding (as used herein) an IAP polypeptide.

By "transgene" is meant any piece of DNA which is inserted by artifice into a cell, and becomes part of the genome of the organism which develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (*i.e.*, foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism.

By "transgenic" is meant any cell which includes a DNA sequence which is inserted by artifice into a cell and becomes part of the genome of the organism which develops from that cell. As used herein, the transgenic organisms are generally transgenic mammalian (*e.g.*, rodents such as rats or mice) and the DNA (transgene) is inserted by artifice into the nuclear genome.

By "transformation" is meant any method for introducing foreign molecules into a cell. Lipofection, calcium phosphate precipitation, retroviral delivery, electroporation, and biolistic transformation are just a few of the teachings which may be used. For example, biolistic transformation is a method for introducing foreign molecules into a cell using velocity driven microprojectiles such as tungsten or gold particles. Such velocity-driven methods originate from pressure bursts which include, but are not limited to, helium-driven, air-driven, and gunpowder-driven techniques. Biolistic transformation may be applied to the transformation or transfection of a wide variety of cell types and intact tissues including, without limitation, intracellular organelles (*e.g.*, and mitochondria and chloroplasts), bacteria, yeast, fungi, algae, animal tissue, and cultured cells.

By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (*i.e.*, facilitates the production of, *e.g.*, an IAP polypeptide, a recombinant protein or a RNA molecule).

By "reporter gene" is meant a gene whose expression may be assayed; such genes include, without limitation, glucuronidase (GUS), luciferase, chloramphenicol transacetylase (CAT), and β-galactosidase.

By "promoter" is meant minimal sequence sufficient to direct transcription. Also included in the invention are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell type-specific, tissue-specific or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the native gene.

By "operably linked" is meant that a gene and one or more regulatory sequences are connected in such a way as to permit gene expression when the appropriate molecules (*e.g.*, transcriptional activator proteins are bound to the regulatory sequences).

By "conserved region" is meant any stretch of six or more contiguous amino acids exhibiting at least 30%, preferably 50%, and most preferably 70% amino acid sequence identity between two or more of the IAP family members, (*e.g.*, between human HIAP-1, HIAP-2, and XIAP). Examples of preferred conserved regions are shown (as boxed or designated sequences) in Figures 5-7 and Tables 1 and 2, and include, without limitation, BIR domains and ring zinc finger domains.

By "detectably-labelled" is meant any means for marking and identifying the presence of a molecule, *e.g.*, an oligonucleotide probe or primer, a gene or fragment thereof, or a cDNA molecule. Methods for detectably-labelling a molecule are well known in the art and include, without limitation, radioactive labelling (*e.g.*, with an isotope such as $^{32}P$ or $^{35}S$) and nonradioactive labelling (*e.g.*, chemiluminescent labelling, *e.g.*, fluorescein labelling).

By "antisense," as used herein in reference to nucleic acids, is meant a nucleic acid sequence, regardless of length, that is complementary to a region on the coding strand of nucleic acid molecule (*e.g.*, genomic DNA, cDNA, or mRNA) that encodes an IAP or a NAIP polypeptide. The region of the nucleic acid molecule encoding an IAP or a NAIP polypeptide that the antisense molecule is complementary to may be a region within the coding region, a region upstream of the coding region, a region downstream of the coding

region, or a region within an intron, where the nucleic acid molecule is genomic DNA. Preferably, the antisense nucleic acid is capable of enhancing apoptosis when present in a cell which normally does not undergo sufficient apoptosis and/or is between 8 and 25 nucleotides in length. Preferably, the increase is at least 10%, relative to a control, more preferably 25%, and most preferably 1-fold or more. It will be understood that antisense nucleic acid molecules may have chemical modifications known in the art of antisense design to enhance antisense compound efficiency.

By "purified antibody" is meant antibody which is at least 60%, by weight, free from proteins and naturally occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, antibody, *e.g.*, an IAP specific antibody. A purified antibody may be obtained, for example, by affinity chromatography using recombinantly-produced protein or conserved motif peptides and standard techniques.

By "specifically binds" is meant an antibody that recognizes and binds a protein but that does not substantially recognize and bind other molecules in a sample, *e.g.*, a biological sample, that naturally includes protein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

## Brief Description of the Drawings

Fig. 1 is the human XIAP cDNA sequence (SEQ ID NO: 3) and the XIAP polypeptide sequence (SEQ ID NO: 4).

Fig. 2 is the human HIAP-1 cDNA sequence (SEQ ID NO: 5) and the HIAP-1 polypeptide sequence (SEQ ID NO: 6).

Fig. 3 is the human HIAP-2 cDNA sequence (SEQ ID NO: 7) and the HIAP-2 polypeptide sequence (SEQ ID NO: 8).

Fig. 4 is the murine XIAP (also referred to as "MIAP-3" or "m-XIAP") cDNA sequence (SEQ ID NO: 9) and encoded murine XIAP polypeptide sequence (SEQ ID NO: 10).

Fig. 5 is the murine HIAP-1 (also referred to as "MIAP-1" or "m-HIAP-1") cDNA sequence (SEQ ID NO: 11) and the encoded murine HIAP-1 polypeptide sequence (SEQ ID NO: 12).

Fig. 6 is the murine HIAP-2 (also referred to as "MIAP-2"or "m-HIAP-2") cDNA sequence (SEQ ID NO: 13) and the encoded murine HIAP-2 polypeptide (SEQ ID NO: 14).

Fig. 7 is a photograph of a Northern blot illustrating human HIAP-1 and HIAP-2 mRNA expression in human tissues.

Fig. 8 is a photograph of a Northern blot illustrating human HIAP-2 mRNA expression in human tissues.

Fig. 9 is a photograph of a Northern blot illustrating human XIAP mRNA expression in human tissues.

Figs. 10A - 10D are graphs depicting suppression of apoptosis by XIAP, HIAP-1, HIAP-2, BCL-2, SMN, and 6-MYC.

Fig. 11 is a photograph of an agarose gel containing cDNA fragments that were amplified, with HIAP 1-specific primers, from RNA obtained from Raji, Ramos, EB-3, Burkitt's lymphoma cells, and Jiyoye cells, and cells from normal placenta.

Fig. 12 is a photograph of a Western blot containing protein extracted from Jurkat and astrocytoma cells stained with an anti-XIAP antibody. The position and size of a series of marker proteins is indicated.

Fig. 13 is a photograph of a Western blot containing protein extracted from Jurkat cells following treatment as described in Example XII. The blot was stained with a rabbit polyclonal anti-XIAP antibody. Lane 1, negative control; lane 2, anti-Fas antibody; lane 3, anti-Fas antibody and cycloheximide; lane 4, TNF-α; lane 5, TNF-α and cycloheximide.

Fig. 14 is a photograph of a Western blot containing protein extracted from HeLa cells following exposure to anti-Fas antibodies. The blot was stained with a rabbit polyclonal anti-XIAP antibody. Lane 1, negative control; lane 2, cycloheximide; lane 3, anti-Fas antibody; lane 4, anti-Fas antibody and cycloheximide; lane 5, TNF-α; lane 6, TNF-α and cycloheximide.

Figs. 15A and 15B are photographs of Western blots stained with rabbit polyclonal anti-XIAP antibody. Protein was extracted from HeLa cells (Fig. 15A) and Jurkat cells (Fig. 15B) immediately, 1, 2, 3, 5, 10, and 22 hours after exposure to anti-Fas antibody.

Figs. 16A and 16B are photographs of Western blots stained with an anti-CPP32 antibody (Fig. 16A) or a rabbit polyclonal anti-XIAP antibody (Fig. 16B). Protein was extracted from Jurkat cells immediately, 3 hours, or 7 hours after exposure to an anti-Fas antibody. In addition to total protein, cytoplasmic and nuclear extracts are shown.

Fig. 17 is a photograph of a polyacrylamide gel following electrophoresis of the products of an *in vitro* XIAP cleavage assay.

Figs. 18 and 19 shows the increased level of HIAP-1 and HIAP-2 mRNA, respectively, in breast cancer cell lines having p53 mutations (lanes 5-7). The bottom portion of the figure shows the control.

Fig. 20 shows the influence of Taxol on DNA fragmentation in Cisplatin-sensitive (right) and resistant (left) human ovarian epithelial cancer cells.

Fig. 21 shows the influence of Cisplatin on DNA fragmentation in sensitive (right) and resistant (left) human ovarian epithelial cancer cells.

Fig. 22 shows the effects of Taxol on XIAP and HIAP-2 protein levels in Cisplatin sensitive (right) and resistant (left) human ovarian epithelial cancer cells.

Figs. 23A and 23B show the influence of Taxol and TGFβ on HIAP-2 mRNA levels in Cisplatin sensitive (right) and resistant (left) human epithelial cancer cells.

Figs. 24A and 24B show the effect of TGFβ on XIAP protein expression (Fig. 24A) and DNA fragmentation (Fig. 24B) in Cisplatin-sensitive (OV2008) and cisplatin-resistant (C13) cells.

Fig. 25 is a series of bar graphs showing the effect of XIAP and HIAP-2 down-regulation on ovarian epithelial cancer cell viability and number. The top two panels show dead cells as a percentage of total cell population. The bottom two panels illustrate total cell number at the end of the infection period. Data represents the mean +/- SEM of four experiments. **$p<0.01$, ***$p<0.001$ (compared to vector control).

Fig. 26A is a set of photographs showing the influence of XIAP down-regulation on whole cell morphology (phase contrast; black arrows indicate cell detachment) in OV2008 cells after 60 hours of adenovirus infection with vector only (left) or adenoviral antisense XIAP (right). MOI=5 (1X; "a" and "b"); magnification 400X.

Fig. 26B is a series of photographs ("a" through "d") showing the influence of XIAP down-regulation on nuclear morphology (Hoechst staining; white arrows show nuclear

fragmentation) in OV2008 cells after 60 hours of adenovirus infection with vector only ("a" and "c") or adenoviral antisense XIAP ("b" and "d"). MOI=5 (1X; "a" and "b") and MOI=10 (2X; "c" and "d"); magnification 400X.

Fig. 26C is a bar graph showing the influence of XIAP down-regulation on the extent of apoptosis in OV2008 cells after 60 hours of no treatment, adenovirus infection with vector only, or adenovirus infection with antisense XIAP. Data represents the mean +/- SEM of three to four experiments. MOI=5 (1X) and MOI=10 (2X); *p<0.05, **p<0.01 (compared to vector control).

Fig. 26D is a representative Western blotting analysis showing effective XIAP antisense infection in OV2008 cells after 60 hours of no treatment, adenovirus infection with vector only, or adenovirus infection with antisense XIAP. Lanes are, from left to right: control, vector (1X), vector (2X), antisense XIAP (1X), and antisense XIAP (2X). MOI=5 (1X) and MOI=10 (2X).

Fig. 26E is a bar graph showing changes in XIAP protein content in OV2008 cells after 60 hours of no treatment, adenovirus infection with vector only, or adnovirus infection with antisense XIAP, as analyzed densitometrically, using a Molecular Dynamic Phosphoimager. Data represents the mean +/- SEM of three to four experiments. MOI=5 (1X) and MOI=10 (2X); *p<0.05, **p<0.01 (compared to vector control).

Fig. 27A is a series of photographs showing effects of cisplatin-induced apoptosis (at 0 and 30 μM cisplatin in a 24 hour culture) the nuclear morphology of cisplatin-sensitive cells (OV2008; left two photographs) and cisplatin-resistant cells (C13; right two photographs), using Hoechst staining, magnification 400X; arrows show fragmented nuclei.

Fig. 27B is a set of photographs showing agarose gel immobilized electrophoretically resolved apoptotic low molecular weight DNA fragmentation from cisplatin treated OV2008 and C13 cells.

Fig. 27C is a line graph showing a concentration-response study of apoptosis in OV2008 and C13 cells following 24 hours of culture in 0, 10, 20, and 30 μM cisplatin. Data represents the mean +/- SEM of three experiments. **p<0.01 (compared to control).

Fig. 28A is a series of representative Western blotting analyses showing concentration-dependent inhibition of XIAP and HIAP-2 protein expression in cisplatin-sensitive (OV2008) and cisplatin-resistant (C13) ovarian epithelial cancer cells following 24

hour culture with 0, 10, 20, and 30 μM cisplatin. Equal amounts of solubilized proteins (20-60 μg/lane, depending on the individual experiment) were analyzed by Western blot using anti-human XIAP or anti-HIAP-2 antibodies.

Fig. 28B is a panel of bar graphs showing the changes in XIAP (left two graphs) and HIAP-2 (right two graphs) protein content as analyzed densitometrically, using the Molecular Dynamics Phosphoimager, for cisplatin-treated (24 hours at indicated concentration) OV2008 cells (upper two graphs) and C13 cells (lower two graphs). Data represents the mean +/- SEM of three experiments. *$p<0.05$, **$p<0.01$ (compared to control).

Fig. 29A is a series of representative Western blotting analyses showing concentration-dependent inhibition of XIAP and HIAP-2 protein expression in cisplatin-sensitive (OV2008) and cisplatin-resistant (C13) ovarian epithelial cancer cells following 6, 12, or 24 hours of culture with or without 30 μM cisplatin. Equal amounts of solubilized proteins (20-60 μg/lane, depending on the individual experiment) were analyzed by Western blot using anti-human XIAP or anti-HIAP-2 antibodies.

Fig. 29B is a panel of bar graphs showing the changes in XIAP (left two graphs) and HIAP-2 (right two graphs) protein content as analyzed densitometrically, using the Molecular Dynamics Phosphoimager, for OV2008 cells (white bars) and C13 cells (black bars) cultured with or without 30 μM cisplatin for 6, 12, or 24 hours. Data represents the mean +/- SEM of three experiments. *$p<0.05$, **$p<0.01$ (compared to control).

Fig. 30A is a series of representative Western blotting analyses showing concentration-dependent inhibition of XIAP and HIAP-2 protein expression in cisplatin-sensitive (A2780s) and cisplatin-resistant (A2780cp) ovarian epithelial cancer cells following hours of culture with or without 30 μM cisplatin. Equal amounts of solubilized proteins (40-60 μg/lane, depending on the individual experiment) were analyzed by Western blot using anti-human XIAP or anti-HIAP-2 antibodies.

Fig. 30B is a panel of bar graphs showing the changes in XIAP (top graph) and HIAP-2 (bottom graph) protein content as analyzed densitometrically, using the Molecular Dynamics Phosphoimager, for A2780s cells (left) and A2780cp cells (right) cultured with (black bars) or without (white bars) 30 μM cisplatin for 24 hours. Data represents the mean +/- SEM of three experiments. **$p<0.01$ (compared to control).

Fig. 31A is set of photographs ("a" through "d") showing the effects of XIAP overexpression on the apoptotic action of cisplatin (30μM) on nuclear morphology of cisplatin-sensitive OV2008 cells after 48 hours of infection of these cells with adenoviral sense XIAP cDNA or vector only (control). At a magnification of 400X. "a", vector (no cisplatin); "b", sense XIAP (no cisplatin); "c", vector plus cisplatin-treatment; "d", sense XIAP plus cisplatin treatment.

Fig. 31B is a graph showing the percentage of total cell population undergoing apoptosis of 30 μM cisplatin-treated OV2008 cells following 48 hours of infection of these cells with adenoviral sense XIAP cDNA or vector only (control). Data represent mean +/- SEM of three experiments. *$p<0.05$, ***$p<0.001$ (compared to vector control); $^{--}p<0.01$, $^{---}p<0.001$ (compared to vector plus cisplatin group).

Fig. 31C is a representative Western blotting analysis showing changes in XIAP protein content in OV2008 cells following infection with adenoviral sense XIAP cDNA or vector only (control) with or without treatment with 30 μM cisplatin. Lanes are, from left to right: control, vector, vector plus cisplatin, sense XIAP, and sense XIAP plus cisplatin.

Fig. 31D is a graph showing the changes in XIAP protein content in OV2008 cells following infection with adenoviral sense XIAP cDNA or vector only (control) with or without treatment with 30 μM cisplatin, as analyzed densitometrically, using the Molecular Dynamic Phosphoimager. Data represent mean +/- SEM of three experiments. $^{*}p<0.05$, $^{***}p<0.001$ (compared to vector control); $^{--}p<0.01$, $^{+++}p<0.001$ (compared to vector + cisplatin group).

Figs. 32A-32D are a series of photographs showing the *in situ* detection of apoptosis (using TUNEL) and immunolocalization of PCNA, XIAP and HIAP-2 in human ovarian surface epithelial tumour tissue. Fig. 32A indicates the *in situ* TUNEL localization of apoptotic cells. Figs. 32B, 32C, and 32D represent immuno-reactivates for PCNA, XIAP and HIAP-2, respectively. The regions of tumor shown in the circle and the rectangle in each of Figs. 32A-32D was TUNEL-positive and TUNEL-negative, respectively. Magnification is 400X.

## Detailed Description

Previously, we have provided a novel family of inhibitors of apoptosis, the IAPs, and an additional related anti-apoptotic protein, NAIP. Here we provide identification of cancer types in which dysregulation of the IAPs and NAIP is apparent. Our results are of paramount importance and provide diagnostics, prognostics, treatments, and drug screens aimed at the detection and effective treatment of cancer.

Cancer Screening

We initially studied IAP and NAIP expression levels in a variety of normal tissues and cancer cell lines using commercially available northern blots. Elevated XIAP, HIAP-1 and HIAP-2 mRNA was noted in a surprising number of cancer lines of diverse lineage, including colorectal cancer, lymphoma, leukemia, and melanoma cell lines. In contrast, BCL-2 mRNA was elevated in only a single cell line. Although this result reinforced the importance of the IAPs and NAIP in cancer, the question remained as to whether the individual cancer cell lines on the blot were representative of the cancer type. As a result, we screened panels of cancer cell lines of particular tumor type by northern blot and quantitative RT-PCR analysis in order to ascertain the frequency of IAP and NAIP dysregulation. The results are summarized as follows:

*Burkitt's Lymphoma.*

We studied both the frequency and consequences of IAP upregulation in Burkitt's lymphoma. Elevated levels of HIAP-1 and HIAP-2 have been found in the vast majority of the Burkitt's cell lines examined. Furthermore, those Burkitt's lines expressing low levels of HIAP-1 are transcriptionally activated by Epstein-Barr virus (EBV) infection.

*Breast Adenocarcinoma.*

A key observation was made in this survey, in which a correlation was observed between drug resistance, p53 status, and HIAP-1 and HIAP-2 expression. Four of the cell lines possessed wild-type p53, while three possessed documented p53 mutations that correlated with resistance to the anti-cancer drug adriamycin. Significantly, the three lines which were relatively more drug resistant also displayed elevated HIAP-1 and HIAP-2

mRNA levels. These results indicate that one of the ways that p53 controls apoptosis is through regulation of these genes.

*Ovarian Carcinoma.*

mRNA *in situ* analysis suggest a role for NAIP in the developmental biology of the ovary. Overexpression of HIAP-2 and XIAP mRNA has also been documented in some ovarian cancer cell lines.

*Pancreatic Cancer.*

Approximately 25% of the pancreatic cancer cell lines tested to date demonstrate HIAP-1 and HIAP-2 mRNA elevation.

*Summary of Cancer Panels.*

To date, a significant fraction of cancer cell lines of each type examined display elevated IAP levels. Increased NAIP levels are also implicated in cancer. Our results indicate that HIAP-1 and HIAP-2 tend to be the most frequently and dramatically upregulated. The apparent coordinate regulation of both genes was surprising given that the normal tissue distribution of these proteins is very different. Our observations are strengthened by the fact that HIAP-1 and HIAP-2 reside in tandem array on chromosome 11q23, a site frequently rearranged in lymphomas and leukemias.

**Transcriptional regulation of the IAPs in cancer cell lines.**

Our experiments have established a correlation between p53 status and transcriptional overexpression of HIAP-1 and HIAP-2. This provides an important new way in which to enhance apoptosis, particularly in view of the fact that the mechanism by which p53 controls cell fate remains largely unknown. It has previously been documented that wild-type p53 negatively down-regulates BCL-2, and positively upregulates the BCL-2 antagonist BAX. In some cancer cell types, mutation of p53 causes a two-fold effect; namely, the upregulation of BCL-2, and down regulation of BAX, both of which contribute to the anti-apoptotic phenotype. While not wishing to bind ourselves to a particular theory, we believe that wild-type p53 also transcriptionally suppresses HIAP-1 and HIAP-2. DNA damage that includes

the increase in wild-type levels p53 levels would therefore result in decreased HIAP-1 and HIAP-2 in normal cells, resulting in apoptosis. Mutations in the p53 gene would therefore result in a loss of transcriptional control of these IAP genes. As a result, p53 mutant cancer cells would display constitutively high levels of HIAP-1 and HIAP-2, rendering the cells resistant to anti-cancer therapies. The p53/HIAP-1 and HIAP-2 correlations may be extended to the other cancer cell line panels. One may directly demonstrate p53 regulation of the IAPs using transfection assays and northern blot analysis.

Accordingly, we predict that cancer cells having p53 mutations (p53*) will have increased IAP levels resulting in a poor response to chemotherapeutics. Because IAP levels may be assessed more readily than the presence of a p53* mutation, our discovery also provides an important improvement in cancer diagnosis and prognosis (see below).

**Transgenic Mice**

We have constructed a number of IAP and NAIP transgenic mouse expression vectors, including T-cell, B-cell, and neuronal specific promoter constructs. Founder mice have been identified and are viable, and, for most of these constructs, we have developed breeding colonies. These mice will likely be prone to cancers of the tissue types in which the promoter is active. Thus the mice provide an excellent resource for testing the efficacy of anti-sense oligonucleotides and for screening for apoptosis-enhancing cancer therapeutics. Standard mouse drug screening models and gene delivery protocols may be employed to utilize the mice for this purpose.

**Diagnostic/Prognostic Reagents**

There is a relative lack of diagnostic and prognostic tests which clinical oncologists may utilize in determining the appropriate degree of intervention in the treatment of cancer. Mutation of the p53 gene remains one of the best prognostic indicators in cancer biology. However, the number of different mutations identified to date is great and the mutations are scattered throughout the gene. In addition, many mutations in p53 result in an inappropriate stabilization of the protein, which allows detection at the protein level rather than at the mRNA level. Mutations which alter the transactivation/repression activities of the protein are not necessarily apparent at either the mRNA or protein levels. On the other hand, if IAP

and NAIP expression levels correlate with p53 mutation they may provide more valuable prognostic information and assist in the determination of which patients require more aggressive treatment or which patients are, perhaps, not treatable with currently approved therapies. This latter class of patients may be identified as ideal candidates for clinical testing of new cancer therapeutics, particularly those which decrease IAP levels or act in a manner independent of the anti-apoptotic pathway.

Thus, the invention provides at least two assays for prognosis an diagnosis. Semi-quantitative RT-PCR based assays may be used to assay for IAP and/or NAIP gene or protein expression levels. Alternatively, monoclonal antibodies may be incorporated into an ELISA (enzyme-linked immunosorbent assay) -type assay for direct determination of protein levels.

**Therapeutic Products**

For IAP or NAIP-related therapies, one may employ the paradigms utilized for BCL-2 and RAS antisense development, although, in contrast to RAS antisense, accommodation of mutations is not required. Most useful are antisense constructs which enhance apoptosis at least 10%, preferably by enhancing degradation of the RNA in the nucleus.

In addition to the antisense approaches described herein, the invention features small molecule screening assays which may be used to identify lead compounds that negatively regulate the IAPs or NAIP. For example, compounds which enhance apoptosis in the presence of IAP overexpression or which decrease the level of IAP biological activity may be detected and are useful cancer therapeutics.

Molecules that are found, by the methods described herein, to effectively modulate IAP gene expression or polypeptide activity may be tested further in standard animal cancer models. If they continue to function successfully in an *in vivo* setting, they may be used as therapeutics to either inhibit or enhance apoptosis, as appropriate.

*Manipulation of cancer chemotherapeutic drug resistance using an antisense oligonucleotide and fragment approaches.*

We have documented that overexpression of the IAPs renders cell lines resistant to serum growth factor withdrawal, tumor necrosis factor alpha (TNF) and menadione exposure, all of which are treatments that normally induce apoptosis. Herein we describe the extension

of these studies to cancer cell lines using apoptotic triggers used in clinical situations, such as doxorubicin, adriamycin, and methotrexate. Our findings have led up to design antisense RNA therapeutics. Rapid screening of multiple cell lines for apoptotic response has been made feasible through the generation of a series of sense and antisense adenoviral IAP and NAIP expression vectors, as well as control lacZ viruses. One may now show enhanced drug resistance or enhanced drug sensitivity using these expression constructs. In addition, antisense adenovirus constructs have been developed and used to test reversal of the drug resistant phenotype of appropriate cell lines.

We have surveyed cancer cell lines with the objective of identifying tumor types in which IAP or NAIP overexpression is apparent or altered and these results are described both above and in the Examples below. Concomitant to this research, we have designed a series of antisense oligonucleotides to various regions of each of the IAPs. After testing in an assay system, *i.e.*, with the adenoviral vectors system, these oligonucleotides, as well as antisense oligonucleotides to various regions of NAIP, may be used to enhance drug sensitivity. Animal modeling of the effectiveness of antisense IAP and NAIP oligonucleotides may also be employed as a step in testing and appropriate transgenic mammals for this are described above and also generally available in the art.

The following describes some of the testing systems which may be employed.

**Anti-Cancer Gene Therapy**

Retroviral vectors, adenoviral vectors, adeno-associated viral vectors, or other viral vectors with the appropriate tropism for cells requiring enhanced apoptosis (for example, breast cancer and ovarian cancer cells) may be used as an oligonucleotide transfer delivery system for a therapeutic constructs.

Alternatively, standard non-viral delivery methods may be used. Numerous vectors useful for viral delivery are generally known (Miller, A.D., Human Gene Therapy 1: 5-14, 1990; Friedman, T., Science 244: 1275-1281, 1989; Eglitis and Anderson, BioTechniques 6: 608-614, 1988; Tolstoshev and Anderson, Curr. Opin. Biotech. 1: 55-61, 1990; Cornetta *et al.*, Prog. Nucl. Acid Res. and Mol. Biol. 36: 311-322, 1987; Anderson, W. F., Science 226: 401-409, 1984; Moen, R. C., Blood Cells 17: 407-416, 1991; Miller *et al.*, BioTechniques 7:

980-990, 1989; Le Gal La Salle *et al.*, Science 259: 988-990, 1993; and Johnson, Chest 107: 77S-83S, 1995).

Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg *et al.*, New Engl. J. Med. 323: 570-578, 1990; Anderson *et al.*, U.S. Patent No. 5,399,346).

Non-viral approaches may also be employed for the introduction of therapeutic nucleic acid molecules (*e.g.*, oligonucleotides) into cells otherwise predicted to undergo apoptosis. For example, IAP may be introduced into a neuron or a T cell by lipofection (Felgner *et al.*, Proc. Natl. Acad. Sci. USA 84: 7413-7417, 1987; Ono *et al.*, Neurosci. Lett. 117: 259-263, 1990; Brigham *et al.*, Am. J. Med. Sci. 298: 278-281, 1989; Staubinger *et al.*, Meth. Enz. 101: 512-527, 1983), asialorosonucoid-polylysine conjugation (Wu *et al.*, J. Biol. Chem. 263: 14621-14624, 1988; Wu *et al.*, J. Biol. Chem. 264: 16985-16987, 1989); direct deliver in saline; or, less preferably, microinjection under surgical conditions (Wolff *et al.*, Science 247: 1465-1468, 1990).

For any of the methods of application described above, the therapeutic nucleic acid construct is preferably applied to the site of the needed apoptosis event (for example, by injection). However, it may also be applied to tissue in the vicinity of the predicted apoptosis event, to a blood vessel supplying the cells predicted to require enhanced apoptosis, or orally.

In the constructs described, nucleic acid expression can be directed from any suitable promoter (*e.g.*, the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element. For example, if desired, enhancers known to preferentially direct gene expression in ovarian cells, breast tissue, neural cells, T cells, or B cells may be used to direct expression. The enhancers used could include, without limitation, those that are characterized as tissue- or cell-specific in their expression. Alternatively, if a clone used as a therapeutic construct, regulation may be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

Anti-cancer therapy is also accomplished by direct administration of the therapeutic sense IAP nucleic acid or antisense IAP nucleic acid (*e.g.*, oligonucleotides) to a cell that is expected to require enhanced apoptosis. The nucleic acid molecule may be produced and

isolated by any standard technique, but is most readily produced by *in vitro* transcription using an IAP related nucleic acid under the control of a high efficiency promoter (*e.g.*, the T7 promoter), or, by organic synthesis techniques (for, *e.g.*, oligonucleotides). Administration of IAP antisense nucleic acid to malignant cells can be carried out by any of the methods for direct nucleic acid administration described above, or any method otherwise known in the art.

Another therapeutic approach within the invention involves administration of recombinant IAP protein fragments or IAP antibodies, either directly to the site where enhanced apoptosis is desirable (for example, by injection) or systemically (for example, by any conventional recombinant protein administration technique).

The dosage of a NAIP or an IAP protein, a polypeptide fragment thereof, a mutant thereof, or antibodies that specifically bind NAIP or an IAP polypeptide depends on a number of factors, including the size and health of the individual patient, but, generally, between O.1 mg and 500 mg inclusive are administered per day to an adult in any pharmaceutically acceptable formulation.

**Administration of IAP and NAIP Polypeptides, Nucleic Acids, and Inhibitors of IAP or NAIP Synthesis or Function**

An IAP or NAIP mutant protein or protein fragment, a nucleic acid molecule encoding the same, a nucleic acid molecule encoding an IAP or NAIP antisense nucleic acid, or a inhibitor of an IAPs or NAIP may be administered within a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the compounds to patients suffering from a disease that is caused by excessive cell proliferation. Administration may begin before the patient is symptomatic.

Any appropriate route of administration may be employed, for example, administration may be parenteral, intravenous, intraarterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intrathecal, intracapsular, intracisternal, intraperitoneal, intranasal, aerosol, suppository, or oral administration. For example, therapeutic formulations may be in the form of liquid solutions or suspensions; for oral

administration, formulations may be in the form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

Methods well known in the art for making formulations are found, for example, in Remington's Pharmaceutical Sciences, (18th edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton, PA. Formulations for parenteral administration may, for example, contain excipients, sterile water, or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, or hydrogenated napthalenes. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for IAP or NAIP modulatory compounds include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

If desired, treatment with an IAP or NAIP mutant proteins or IAP or NAIP fragments, related genes, or other modulatory compounds may be combined with more traditional therapies for the proliferative disease such as surgery or chemotherapy.

**Detection of Conditions Involving Insufficient Apoptosis**

IAP and NAIP polypeptides and nucleic acid sequences find diagnostic use in the detection or monitoring of conditions involving insufficient levels of apoptosis, *i.e.*, proliferative disease. For example, increased expression of IAPs or NAIP, alterations in localization, and IAP or NAIP cleavage correlate with inhibition of apoptosis and cancer in humans. Accordingly, an increase in the level of IAP or NAIP production may provide an indication of a proliferative condition or a predisposition to such a condition. Levels of IAP or NAIP expression may be assayed by any standard technique. For example, IAP or NAIP expression in a biological sample (*e.g.*, a biopsy sample) may be monitored by standard Northern blot analysis or may be aided by PCR (see, *e.g.*, Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1994; PCR Technology: Principles and

Applications for DNA Amplification, H.A. Ehrlich, Ed., Stockton Press, NY; Yap *et al.*, Nucl. Acids. Res. 19: 4294, 1991).

Alternatively, a biological sample obtained from a patient may be analyzed for one or more mutations in the IAP or NAIP sequences or p53 sequences using a mismatch detection approach. Generally, these techniques involve PCR amplification of nucleic acid from the patient sample, followed by identification of the mutation (*i.e.*, mismatch) by either altered hybridization, aberrant electrophoretic gel migration, binding or cleavage mediated by mismatch binding proteins, or direct nucleic acid sequencing. Any of these techniques may be used to facilitate mutant IAP or NAIP detection, and each is well known in the art; examples of particular techniques are described, without limitation, in Orita *et al.*, Proc. Natl. Acad. Sci. USA 86: 2766-2770, 1989; Sheffield *et al.*, Proc. Natl. Acad. Sci. USA 86: 232-236, 1989).

In yet another approach, immunoassays are used to detect or monitor IAP or NAIP protein in a biological sample. IAP or NAIP-specific polyclonal or monoclonal antibodies (produced as described above) may be used in any standard immunoassay format (*e.g.*, ELISA, Western blot, or RIA) to measure IAP or NAIP polypeptide levels from cancerous control cells. These levels would be compared to wild-type IAP or NAIP levels, with a decrease in IAP production relative to a wild-type cell indicating a condition involving increased apoptosis and a decrease relative to a known cancer cell indicating a decreased likelihood of an IAP or NAIP-related cancer. Examples of immunoassays are described, *e.g.*, in Ausubel *et al.*, *supra*. Immunohistochemical techniques may also be utilized for IAP or NAIP detection. For example, a tissue sample may be obtained from a patient, sectioned, and stained for the presence of IAP or NAIP using an anti-IAP or anti-NAIP antiboies and any standard detection system (*e.g.*, one which includes a secondary antibody conjugated to horseradish peroxidase). General guidance regarding such techniques can be found in, *e.g.*, Bancroft and Stevens (Theory and Practice of Histological Technigues, Churchill Livingstone, 1982) and Ausubel *et al.* (*supra*).

In one preferred example, a combined diagnostic method may be employed that begins with an evaluation of IAP or NAIP protein production (for example, by immunological techniques or the protein truncation test (Hogerrorst *et al.*, Nature Genetics 10:208-212, 1995)) and also includes a nucleic acid-based detection technique designed to

identify more subtle IAP or NAIP alterations, *e.g.*, mutations. As described above, a number of mismatch detection assays are available to those skilled in the art, and any preferred technique may be used. Mutations in IAP or NAIP may be detected that either result in enhanced IAP or NAIP expression or alterations in IAP or NAIP biological activity. In a variation of this combined diagnostic method, IAP or NAIP biological activity is measured as anti-apoptotic activity using any appropriate apoptosis assay system (for example, those described above).

Mismatch detection assays also provide an opportunity to diagnose an IAP-mediated or an NAIP-mediated predisposition to diseases caused by insufficient apoptosis. For example, a patient heterozygous for an IAP or a NAIP mutation may show no clinical symptoms and yet possess a higher than normal probability of developing one or more types of proliferative diseases. Given this diagnosis, a patient may take precautions to minimize their exposure to adverse environmental factors (for example, UV exposure or chemical mutagens) and to carefully monitor their medical condition (for example, through frequent physical examinations). This type of IAP or NAIP diagnostic approach may also be used to detect IAP or NAIP mutations in prenatal screens. The IAP or NAIP diagnostic assays described above may be carried out using any biological sample (for example, any biopsy sample or bodily fluid or tissue) in which IAP or NAIP is normally expressed. Identification of a mutant IAP or NAIP gene may also be assayed using these sources for test samples.

Alternatively, an alteration in IAP or NAIP activity, particularly as part of a diagnosis for predisposition to IAP-associated or NAIP-associated proliferative disease, may be tested using a nucleic acid sample from any cell, for example, by mismatch detection techniques. Preferably, the DNA sample is subjected to PCR amplification prior to analysis.

The following examples are meant to illustrate, not limit, the invention.

## EXAMPLE 1: ELEVATED IAP LEVELS IN CANCER CELL LINES

In order to specifically demonstrate the utility of IAP gene sequences as diagnostics and prognostics for cancer, a Human Cancer Cell Line Multiple Tissue Northern Blot (Clontech, Palo Alto, CA; #7757-1) was probed. This Northern blot contained approximately 2 μg of poly $A^+$ RNA per lane from eight different human cell lines: (1) promyelocytic leukemia HL-60, (2) HeLa cell S3, (3) chronic myelogenous leukemia K-562, (4)

lymphoblastic leukemia MOLT-4, (5) Burkitt's lymphoma Raji, (6) colorectal adenocarcinoma SW480, (7) lung carcinoma A549, and (8) melanoma G361. As a control, a Human Multiple Tissue Northern Blot (Clontech, Palo Alto, CA; #7759-1) was probed. This Northern blot contained approximately 2 μg of poly $A^+$ RNA from eight different human tissues: (1) spleen, (2) thymus, (3) prostate, (4) testis, (5) ovary, (6) small intestine, (7) colon, and (8) peripheral blood leukocytes.

The Northern blots were hybridized sequentially with: (1) a 1.6 kb probe to the XIAP coding region, (2) a 375 bp HIAP-2 specific probe corresponding to the 3' untranslated region, (3) a 1.3 kb probe to the coding region of HIAP-1, which cross-reacts with HIAP-2, (4) a 1.0 kb probe derived from the coding region of BCL-2, and (5) a probe to β-actin, which was provided by the manufacturer. Hybridization was carried out at 50°C overnight, according to the manufacturer's suggestion. The blot was washed twice with 2X SSC, 0.1% SDS at room temperature for 15 minutes and then with 2X SSC, 0.1% SDS at 50°C.

All cancer lines tested showed increased IAP expression relative to samples from non-cancerous control tissues (Table 1). Expression of XIAP was particularly high in HeLa (S-3), chronic myelogenous leukemia (K-562), colorectal adenocarcinoma (SW-480), and melanoma (G-361) lines. Expression of HIAP-1 was extremely high in Burkitt's lymphoma, and was also elevated in colorectal adenocarcinoma. Expression of HIAP-2 was particularly high in chronic myelogenous leukemia (K-562) and colorectal adenocarcinoma (SW-480). Expression of BCL-2 was upregulated only in HL-60 leukemia cells.

TABLE 1

NORTHERN BLOT IAP RNA LEVELS IN CANCER CELLS*

| | XIAP | HIAP-1 | HIAP-2 |
|---|---|---|---|
| Promyelocytic Leukemia HL-60 | + | + | + |
| Hela S-3 | + | + | + |
| Chronic Myelogenous Leukemia K-562 | +++ | + | +++ |
| Lymphoblastic Leukemia MOLT-4 | +++ | + | + |
| Burkitt's Lymphoma Raji | + | +(x10) | + |
| Colorectal Adenocarcinoma SW-480 | +++ | +++ | +++ |
| Lung Carcinoma A-549 | + | + | + |
| Melanoma G-361 | +++ | + | + |

*Levels are indicated by a (+) and are the approximate increase in RNA levels relative to Northern blots of RNA from non-cancerous control cell lines. A single plus indicates an estimated increase of at least 1-fold

These observations indicate that upregulation of the anti-apoptotic IAP genes may be a widespread phenomenon in proliferative diseases, perhaps occurring much more frequently than upregulation of BCL-2. Furthermore, upregulation may be necessary for the establishment or maintenance of the transformed state of cancerous cells.

In order to pursue the observation described above, *i.e.*, that HIAP-1 is overexpressed in the Raji Burkitt's lymphoma cell line, RT-PCR analysis was performed in multiple Burkitt's lymphoma cell lines. Total RNA was extracted from cells of the Raji, Ramos, EB-3, and Jiyoye cell lines, and as a positive control, from normal placental tissue. The RNA was reverse transcribed, and amplified by PCR with the following set of oligonucleotide primers:

5'-AGTGCGGGTTTTTATTATGTG-3' (SEQ ID NO: 15) and

5'-AGATGACCACAAGGAATAAACACTA-3' (SEQ ID NO: 16), which selectively amplify a hiap-1 cDNA fragment. RT-PCR was conducted using a Perkin Elmer 480 Thermocycler to carry out 35 cycles of the following program: 94°C for 1 minute, 50°C for 1.5 minutes, and 72°C for 1 minute. The PCR reaction product was electrophoresed on an agarose gel and stained with ethidium bromide. Amplified cDNA fragments of the appropriate size were clearly visible in all lanes containing Burkitt's lymphoma samples, but

absent in the lanes containing the normal placental tissue sample, and absent in lanes containing negative control samples, where template DNA was omitted from the reaction (Fig. 11).

**EXAMPLE 2: IAPs IN BREAST CANCER**

The following data relate to the regulation and role of HIAPs in cancer cells. Figs. 18 and 19 show data demonstrating that HIAP-1 and HIAP-2 are both upregulated in breast cancer cell lines that contain mutant p53. The lanes contain 20 µg of total RNA from the following lines: 1. MCF-7 (clone 1, wt p53); 2. MCF-7 (clone 2, wt p53); 3. MCF-7 (American Type Culture Collection, wt p53); 4. MCF-7 (parental line, California, wt p53); 5. MCF-7 (California, adriamycin resistant variant, mutant p53); 6. MDA MB 231 (ATCC, mutant p53, codon 280); 7. T47-D (ATCC, mutant p53, codon 194); 8. ZR-75 (ATCC, wt p53). The amount of RNA loaded on each gel was controlled for by hybridization with glycerol phosphate dehydrogenase (GAPDH).

**EXAMPLE 3: IAPS IN OVARIAN CANCER**

*Overview.*

Epithelial ovarian cancer is the leading cause of death from gynecologic malignancy. Although clinical and histologic prognostic factors such as tumor grade and surgical stage are well understood, the biologic process that leads to uncontrolled cellular growth is less clear. The control of cell numbers during tissue growth is thought to be the results of a balance of cell proliferation and cell death. An aberration in this natural homeostasis likely contributes to malignant cellular transformation.

Recent studies on ovarian cancer cell biology have suggested that the deregulation of apoptosis may be one of the underlying pathologic mechanism in this disease. However, the molecular mechanisms involved in its regulation is poorly understood and the role and regulation of the IAP genes in ovarian cell transformation have not been examined previously. Ovarian epithelial cancer is in part a result of suppressed apoptosis of ovarian surface epithelial cells. The effectiveness of certain chemotherapeutic agents rests on their ability to induce cell death. The loss of responsiveness of the cells to these agents is due to a desensitization of the apoptotic process to these agents. The regulation of ovarian epithelial

cell apoptosis involves changes in the expression of IAP genes and post-translational modification/processing of the IAP gene products.

We have conducted experiments and now believe that IAPs play a key role in maintaining the normal growth of ovarian surface epithelial cells and that the overexpression of these genes leads to cellular transformation. Furthermore, we have discovered that the effectiveness of chemotherapeutic agents in the treatment of this form of malignancy rests upon their ability to suppress the expression of the IAP genes. By seeking to control the regulation of the IAP genes in human ovarian epithelial cancer cells we have provided a rational approach for the development of new chemotherapeutics for patients both responsive and resistant to current cancer drugs. Similarly, assays designed to detect compounds which decrease IAP biological activity provide a rational method for drug discovery.

*Methods.*

a) Human Ovarian Epithelial Cancer Cell Culture

Cisplatin-sensitive (OV2008) and cisplatin-resistant (C13) human ovarian epithelial cells were cultured in a chemically-defined medium at 37°C for up to 48 hours in the presence or absence of TGFβ (20 ng/ml), taxol (0 - 1.0 μM) or cisplatin (0 - 30 μM). At the end of the culture period, cells were either fixed for immunocytochemistry and TUNEL analyses, or snap frozen for subsequent extraction for IAP mRNA and proteins analyses.

b) Identification of Cell Death

For nuclear staining, human ovarian epithelial cancer cells were fixed (4% formalin in PBS; 10 min., room temp.), washed in PBS, resuspended in Hoescht 33248 stain (0.1 μg/ml PBS, 10 min) washed again and spotted onto slides for microscopy. Nuclear staining was observed and photographed using a Zeiss fluorescent microscope equipped with an FITC filter. Apoptotic cells were identified by typical nuclear morphology, and counted using randomly selected fields and numbered photographic slides to avoid bias during counting.

For quantitation of DNA ladders, cellular DNA was extracted using the Qiagen Blood kit (Qiagen Inc., Chatsworth, CA). DNA was quantified by ethidium bromide fluorescence. DNA (0.5μg) was then end labelled by incubating (20 min., room temp.) with Klenow enzyme (2 U in 10 mM Tris plus 5 mM $MgCl_2$) and 0.1 μCi [$\alpha^{32}$P]dCTP. Unincorporated

nucleotides were removed with the Qiagen nucleotide removal kit and samples were resolved by Tris-acetate-EDTA agarose (1.8%) gel electrophoresis. The gel was then dried (2 hours, no heat) and exposed to a Bio-Rad phosphoimager screen to densitometrically quantify low molecular weight DNA (<15 kilo base-pairs), and subsequently to X-ray film at -80°C.

For *in situ* TUNEL labelling of apoptotic cells to identify cell death, the *in situ* cell death detection kit (Boehringer-Mannheim, Indianapolis, IN) was used, according to manufacturer's instructions. Slides prepared for histology were treated (20 min. at 37°C) with terminal transferase in the presence of FITC-conjugated dUTP.

c) Western Blot Analyses for IAPs

Protein extracts were prepared from human surface epithelial cancer cells sonicated (8 sec/cycle, 3 cycles) on ice in sucrose buffer (0.25 M sucrose, 0.025 M NaCl, 1 mM EGTA and 15 mM Tris-HCl pH 6.8, supplemented with 1 mM PMSF, 2 µg/ml of leupeptin and 5 µg/ml of aprotinin. The sonicates were centrifuged at 13,000xg for 10 min., the supernatants were collected and stored at -20°C until electrophoretic analyses were performed. Protein concentration was determined by Bio-Rad Protein Assay. Proteins (10-30 µg) were resolved by one-dimensional SDS-PAGE, and electrophoretically transferred to nitrocellulose membrane. Membranes were blocked with 5% non-fat milk, and subsequently incubated with rabbit polyclonal antibody for IAP [anti-human HIAP-2ΔE (960529; 1:1000 dilution), anti-human NAIP E1.0 (951015; 1:1000 dilution) or anti-human XIAP (1:1000 dilution)] diluted in TBST (10 mM Tris-buffered saline, 0.1% Tween-20, pH7.5) containing 5% milk. An ECL kit was used to visualize immunopositive protein (Amersham Intl., Arlington Heights, IL).

d) Northern Blots for IAP mRNAs

Total RNA from ovarian surface epithelial cancer cells by using RNeasy Kit (Qiagen). The RNA samples (10-15 µg) were quantified spectrophotometrically and size-fractioned by electrophoresis on formaldehyde-agarose gels (1.1%) containing 1 µg/ml ethidium bromide to confirm even loading of RNA samples and adequate separation of 28S and 18S ribosomal bands. The RNAs bands were blotted onto a nylon membrane and cross-linked by UV light. Membranes were prehybridized in 50% formamide, saline sodium citrate

(SSC; 750 mM NaCl, 75 mM sodium citrate), 1X Denhardt's solution, 1% SDS, 4 mM EDTA and 100 µg/ml sheared salmon sperm DNA for 4 hours at 42°C. Hybridization was performed overnight at 42 °C with 20 million cpm of $^{32}$P-labelled IAP cDNA probes (rat NAIP, rat XIAP or human HIAP-2) added to the prehybridization buffer. The membranes were then washed twice with SSC (300 mM NaCl, 30 mM sodium citrate) in 0.1% SDS for 20 min at room temperature and twice with SSC (30 mM NaCl, 3 mM sodium citrate) in 0.1% SDS for 20 min at 55°C and exposed to X-ray film at -80°C for visualization. Densitometric analysis of various IAPs and 28S rRNA band was performed with the Image Analysis Systems from Bio-Rad Laboratories. Data were normalized by the respective 28S and expressed as a percentage of the control (defined as 100%).

*Results*

We observed the following.

1. Cisplatin induced a concentration-dependent increase in the incidence of apoptosis in cisplatin-sensitive (OV2008) but to a lesser extent in -resistant (C13) human ovarian epithelial cells in vitro (Fig. 20). Similarly, Taxol also induced apoptosis in OV2008 cells, but to a lesser extent in the C13 cells (Fig. 21).

2. Basal XIAP and HIAP-2 protein contents were markedly higher in cisplatin-sensitive than -resistant cells. Taxol (0.04-1.0 µM) decreased XIAP and HIAP-2 protein levels in a concentration-dependent manner, the response being more pronounced in sensitive than resistant cells (Fig. 22). A lower molecular weight (approx. 45 kDa) immunoreactive fragment of HIAP-2 was also evident in both the sensitive and resistant cells. The content of this fragment was increased in the C13 cells but decreased in OV2008 cells by Taxol (Fig. 22).

3. Whereas Taxol (0.2 µM) marked suppressed HIAP-2 mRNA abundance in cisplatin-sensitive cells (approx. 80%), it was ineffective in the resistant cells (Fig. 23).

4. TGFβ (20ng/ml) induced apoptosis in OV2008 but not in C13. Although its influence on XIAP protein content in cisplatin-resistant cells was only marginal, it markedly suppressed

the protein level of this IAP in the cisplatin-sensitive cells (Fig. 24A, 24B). TGFβ (20 ng/ml) also decreased HIAP-2 mRNA in OV2008 but not C13 cells (Fig. 23).

***Significant observations and possible applications.***

Induction of apoptosis in human ovarian epithelial cancer cell by Taxol was accompanied by suppressed IAP gene expression. Eventual loss of sensitivity of the cells to the chemotherapeutic agent may be associated with the decreased ability of the cell to express IAP genes. In drug-resistant cells, the decreased HIAP-2 protein content (in the face of an absence of noticeable change in HIAP-2 mRNA abundance) in the presence of Taxol was accompanied an increase in the intensity of a 45 kDa immunoreactive HIAP-2 protein band. These observations lead us to believe that the 45 kDa protein is a proteolytic product of HIAP-2 and plays a role in the development of drug resistance. In addition, the sensitivity of the IAP family in these ovarian cancer cells to Taxol suggest possible novel sites for gene targeting in the development of new chemotherapeutic agents for the treatment of human ovarian epithelial cell cancer.

**EXAMPLE 4: Accumulation of a 26 kDa Cleavage Protein in Astrocytoma Cells**

*Identification of a 26 kDa Cleavage Protein*

A total protein extract was prepared from Jurkat and astrocytoma cells by sonicating them (X3 for 15 seconds at 4°C) in 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM PMSF, 1 μg/ml aprotinin, and 5 mM benzamidine. Following sonication, the samples were centrifuged (14,000 RPM in a micro centrifuge) for five minutes. 20 μg of protein was loaded per well on a 10% SDS-polyacrylamide gel, electrophoresed, and electroblotted by standard methods to PVDF membranes. Western blot analysis, performed as described previously, revealed that the astrocytoma cell line (CCF-STTG1) abundantly expressed an anti-xiap reactive band of approximately 26 kDa, despite the lack of an apoptotic trigger event (Fig. 12). In fact, this cell line has been previously characterized as being particularly resistant to standard apoptotic triggers.

A 26 kDa XIAP-reactive band was also observed under the following experimental conditions. Jurkat cells (a transformed human T cell line) were induced to undergo apoptosis by exposure to an anti-Fas antibody (1 μg/ml). Identical cultures of Jurkat cells were

exposed either to: (1) anti-Fas antibody and cycloheximide (20 μg/ml), (2) tumor necrosis factor alpha (TNF-α, at 1,000 U/ml), or (3) TNF-α and cycloheximide (20 μg/ml). All cells were harvested 6 hours after treatment began. In addition, as a negative control, anti-Fas antibody was added to an extract after the cells were harvested. The cells were harvested in SDS sample buffer, electrophoresed on a 12.5% SDS polyacrylamide gel, and electroblotted onto PVDF membranes using standard methods. The membranes were immunostained with a rabbit polyclonal anti-XIAP antibody at 1:1000 for 1 hour at room temperature. Following four 15 minute washes, a goat anti-rabbit antibody conjugated to horse-radish peroxidase was applied at room temperature for 1 hour. Unbound secondary antibody was washed away, and chemiluminescent detection of XIAP protein was performed. The Western blot revealed the presence of the full-length, 55 kDa XIAP protein, both in untreated and treated cells. In addition, a novel, approximately 26 kDa XIAP-reactive band was also observed in apoptotic cell extracts, but not in the control, untreated cell extracts (Fig. 13).

Cleavage of XIAP occurs in a variety of cell types, including other cancer cell lines such as HeLa. The expression of the 26 kDa XIAP cleavage product was demonstrated in HeLa cells as follows. HeLa cells were treated with either: (1) cyclohexamide (20 μg/ml), (2) anti-Fas antibody (1 μg/ml), (3) anti-Fas antibody (1 μg/ml) and cyclohexamide (20 μg/ml), (4) TNFα (1,000 U/ml), or (5) TNFα (1,000 U/ml) and cyclohexamide (20 μg/ml). All cells were harvested 18 hours after treatment began. As above, anti-Fas antibody was added to an extract after the cells were harvested. HeLa cells were harvested, and the Western blot was probed under the same conditions as used to visualize XIAP-reactive bands from Jurkat cell samples. A 26 kDa XIAP band was again seen in the apoptotic cell preparations (Fig. 14). Furthermore, the degree of XIAP cleavage correlated positively with cellular exposure to apoptotic triggers. Treatment of HeLa cells with cycloheximide or TNFα alone caused only minor apoptosis, and little cleavage product was observed. If the cells were treated with the anti-Fas antibody, a greater amount of cleavage product was apparent. These data indicate that XIAP is cleaved in more than one cell type and in response to more than one type of apoptotic trigger.

*Time Course of Expression*

The time course over which the 26 kDa cleavage product accumulates was examined by treating HeLa and Jurkat cells with anti-Fas antibody (1 μg/ml) and harvesting them either immediately, or 1, 2, 3, 5, 10, or 22 hours after treatment. Protein extracts were prepared and Western blot analysis was performed as described above. Both types of cells accumulated increasing quantities of the 26 kDa cleavage product over the time course examined (Figs. 15A and 15B).

*Subcellular Localization of the 26 kDa XIAP Cleavage Product*

In order to determine the subcellular location of the 26 kDa cleavage product, Jurkat cells were induced to undergo apoptosis by exposure to anti-Fas antibody (1 μg/ml) and were then harvested either immediately, 3 hours, or 7 hours later. Total protein extracts were prepared, as described above, from cells harvested at each time point. In order to prepare nuclear and cytoplasmic cell extracts, apoptotic Jurkat cells were washed with isotonic Tris buffered saline (pH 7.0) and lysed by freezing and thawing five times in cell extraction buffer (50 mM PIPES, 50 mM KCl, 5 mM EGTA, 2 mM $MgCl_2$, 1 mM DTT, and 20 μM cytochalasin B). Nuclei were pelleted by centrifugation and resuspended in isotonic Tris (pH 7.0) and frozen at -80°C. The cytoplasmic fraction of the extract was processed further by centrifugation at 60,000 RPM in a TA 100.3 rotor for 30 minutes. Supernatants were removed and frozen at -80°C. Samples of both nuclear and cytoplasmic fractions were loaded on a 12.5% SDS-polyacrylamide gel, and electroblotted onto PVDF membranes. Western blot analysis was then performed using either an anti-CPP32 antibody (Transduction Laboratories Lexington, KY; Fig. 16A) or the rabbit anti-XIAP antibody described above (Fig. 16B).

The anti-CPP32 antibody, which recognizes the CPP32 protease (also known as YAMA or Apopain) partitioned almost exclusively in the cytoplasmic fraction. The 55 kDa XIAP protein localized exclusively in the cytoplasm of apoptotic cells, in agreement with the studies presented above, where XIAP protein in normal, healthy COS cells was seen to localize, by immunofluoresence microscopy, to the cytoplasm. In contrast, the 26 kDa cleavage product localized exclusively to the nuclear fraction of apoptotic Jurkat cells. Taken together, these observations suggest that the anti-apoptotic component of XIAP could be the 26 kDa cleavage product, which exerts its influence within the nucleus.

*In vitro Cleavage of XIAP protein and Characterization of the Cleavage Product*

For this series of experiments, XIAP protein was labeled with $^{35}S$ using the plasmid pcDNA3-6myc-XIAP, T7 RNA polymerase, and a coupled transcription/translation kit (Promega, Madison, WI) according to the manufacturer's instructions. Radioactively labeled XIAP protein was separated from unincorporated methionine by column chromatography using Sephadex G-50™. In addition, extracts of apoptotic Jurkat cells were prepared following treatment with anti-Fas antibody (1 μg/ml) for three hours. To prepare the extracts, the cells were lysed in Triton X-100 buffer (1% Triton X-100, 25 mM Tris HCl) on ice for two hours and then microcentrifuged for 5 minutes. The soluble extract was retained (and was labeled TX100). Cells were lysed in cell extraction buffer with freeze/thawing. The soluble cytoplasmic fraction was set aside (and labeled CEB). Nuclear pellets from the preparation of the CEB cytoplasmic fraction were solubilized with Triton X-100 buffer, microcentrifuged, and the soluble fractions, which contains primarily nuclear DNA, was retained (and labeled CEB-TX100). Soluble cell extract was prepared by lysing cells with NP-40 buffer, followed by microcentrifugation for 5 minutes (and was labeled NP-40). *In vitro* cleavage was performed by incubating 16 μl of each extract (CEB, TX-100, CEB-TX100, and NP-40) with 4 μl of *in vitro* translated XIAP protein at 37°C for 7 hours. Negative controls, containing only TX100 buffer or CEB buffer were also included. The proteins were separated on a 10% SDS-polyacrylamide gel, which was dried and exposed to X-ray film overnight.

*In vitro* cleavage of XIAP was apparent in the CEB extract. The observed molecular weight of the cleavage product was approximately 36 kDa (Fig. 17). The 10 kDa shift in the size of the cleavage product indicates that the observed product is derived from the amino-terminus of the recombinant protein, which contains six copies of the myc epitope (10 kDa). It thus appears that the cleavage product possesses at least two of the BIR domains, and that it is localized to the nucleus.

## EXAMPLE 5: CHARACTERIZATION OF IAP ACTIVITY AND INTRACELLULAR LOCALIZATION STUDIES

The ability of IAPs to modulate apoptosis can be defined *in vitro* systems in which alterations of apoptosis can be detected. Mammalian expression constructs carrying IAP

cDNAs, which are either full-length truncated, or antisense constructs can be introduced into cell lines such as CHO, NIH 3T3, HL60, Rat-1, or Jurkat cells. In addition, SF21 insect cells may be used, in which case the IAP gene is preferentially expressed using an insect heat shock promoter. Following transfection, apoptosis can be induced by standard methods, which include serum withdrawal, or application of staurosporine, menadione (which induces apoptosis via free radial formation), or anti-Fas antibodies. As a control, cells are cultured under the same conditions as those induced to undergo apoptosis, but either not transfected, or transfected with a vector that lacks an IAP insert. The ability of each IAP related construct to inhibit or enhance apoptosis upon expression can be quantified by calculating the survival index of the cells, *i.e.*, the ratio of surviving transfected cells to surviving control cells. These experiments can confirm the presence of apoptosis inhibiting activity and, as discussed below, can also be used to determine the functional region(s) of an IAP which may be employed to achieve enhancement of apoptosis. These assays may also be performed in combination with the application of additional compounds in order to identify compounds that enhance apoptosis via IAP expression.

## EXAMPLES 6: CELL SURVIVAL FOLLOWING TRANSFECTION WITH IAP CONSTRUCTS AND INDUCTION OF APOPTOSIS

Specific examples of the results obtained by performing various apoptosis suppression assays are shown in Figs. 10A to 10D. For example, CHO cell survival following transfection with one of six constructs and subsequent serum withdrawal is shown in Fig. 10A. The cells were transfected using Lipofectace™ with 2 μg of one of the following recombinant plasmids: pCDNA36myc-xiap (XIAP), pCDNA3-6myc-hiap-1 (HIAP-1), pCDNA3-6myc-hiap-2 (HIAP-2), pCDNA3-bcl-2 (BCL-2), pCDNA3-HA-smn (SMN), and pCDNA3-6myc (6-myc). Oligonucleotide primers were synthesized to allow PCR amplification and cloning of the XIAP, HIAP-1, and HIAP-2 ORFs in pCDNA3 (Invitrogen). Each construct was modified to incorporate a synthetic myc tag encoding six repeats of the peptide sequence MEQKLISEEDL (SEQ ID NO: 17), thus allowing detection of myc-IAP fusion proteins via monoclonal anti-myc antiserum (Egan *et al.*, Nature 363: 45-51, 1993). Triplicate samples of cell lines in 24-well dishes were washed 5 times with serum free media and maintained in serum free conditions during the course of the experiment.

Cells that excluded trypan blue, and that were therefore viable, were counted with a hemocytometer immediately, 24 hours, 48 hours, and 72 hours, after serum withdrawal. Survival was calculated as a percentage of the initial number of viable cells. In this experiment and those presented in Figs. 10B and 10D, the percentage of viable cells shown represents the average of three separate experiments performed in triplicate, +/- average deviation.

The survival of CHO cells following transfection (with each one of the six constructs described above) and exposure to menadione is shown in Fig. 10B. The cells were plated in 24-well dishes, allowed to grow overnight, and then exposed to 20 μM menadione for 1.5 hours (Sigma Chemical Co., St. Louis, MO). Triplicate samples were harvested at the time of exposure to menadione and 24 hours afterward, and survival was assessed by trypan blue exclusion.

The survival of Rat-1 cells following transfection (with each one of the six constructs described above) and exposure to staurosporine is shown in Fig. 10C. Rat-1 cells were transfected and then selected in medium containing 800 μg/ml G418 for two weeks. The cell line was assessed for resistance to staurosporine-induced apoptosis (1 μM) for 5 hours. Viable cells were counted 24 hours after exposure to staurosporine by trypan blue exclusion. The percentage of viable cells shown represents the average of two experiments, +/- average deviation.

The Rat-1 cell line was also used to test the resistance of these cells to menadione (Fig. 10D) following transfection with each of the six constructs described above. The cells were exposed to 10 μM menadione for 1.5 hours, and the NUMBER of viable cells was counted 18 hours later.

## EXAMPLE 7: COMPARISON OF CELL SURVIVAL FOLLOWING TRANSFECTION WITH FULL-LENGTH VS. PARTIAL IAP CONSTRUCTS

In order to investigate the mechanism whereby human IAPs, including XIAP, HIAP-1, and HIAP-2, afford protection against cell death, expression vectors were constructed that contained either: (1) full-length IAP cDNA (as described above), (2) a portion of an IAP gene that encodes the BIR domains, but not the RZF, or (3) a portion of an IAP gene that encodes the RZF, but not the BIR domains. Human and murine XIAP cDNAs were tested by

transient or stable expression in HeLa, Jurkat, and CHO cell lines. Following transfection, apoptosis was induced by serum withdrawal, application of menadione, or application of an anti-Fas antibody. Cell death was then assessed, as described above, by trypan blue exclusion. As a control for transfection efficiency, the cells were co-transfected with a β-gal expression construct. Typically, approximately 20% of the cells were successfully transfected.

When CHO cells were transiently transfected, constructs containing full-length human or mouse xiap cDNAs conferred modest but definite protection against cell death. In contrast, the survival of CHO cells transfected with constructs encoding only the BIR domains (*i.e.*, lacking the RZF domain) was markedly enhanced 72 hours after serum deprivation. Furthermore, a large percentage of cells expressing the BIR domains were still viable after 96 hours, at which time no viable cells remained in the control, *i.e.* non-transfected, cell cultures, and less than 5% of the cells transfected with the vector only, *i.e.*, lacking a cDNA insert, remained viable. Deletion of any of the BIR domains results in the complete loss of apoptotic suppression, which is reflected by a decrease in the percentage of surviving CHO cells to control levels within 72 hours of serum withdrawal.

Stable pools of transfected CHO cells, which were maintained for several months under G418 selection, were induced to undergo apoptosis by exposure to 10 μM menadione for 2 hours. Among the CHO cells tested were those that were stably transfected with: (1) full-length murine XIAP cDNA (MIAP), (2) full-length XIAP cDNA (XIAP), (3) full-length BCL-2 cDNA (BCL-2), (4) cDNA encoding the three BIR domains (but not the RZF) of murine XIAP (BIR), and (5) cDNA encoding the RZF (but not BIR domains) of M-XIAP (RZF). Cells that were non-transfected (CHO) or transfected with the vector only (pcDNA3), served as controls for this experiment. Following exposure to 10 μM menadione, the transfected cells were washed with phosphate buffered saline (PBS) and cultured for an additional 24 hours in menadione-free medium. Cell death was assessed, as described above, by trypan blue exclusion. Less than 10% of the non-transfected or vector-only transfected cells remained viable at the end of the 24 hour survival period. Cells expressing the RZF did not fare significantly better. However, expression of full-length murine XIAP, human XIAP, or BCL-2, and expression of the BIR domains, enhanced cell survival. When the concentration of menadione was increased from 10 μM to 20 μM (with all other conditions

of the experiment being the same as when 10 μM menadione was applied), the percentage of viable CHO cells that expressed the BIR domain cDNA construct was higher than the percentage of viable cells that expressed either full-length murine XIAP or BCL-2.

**EXAMPLE 8: ANALYSIS OF THE SUBCELLULAR LOCATION OF EXPRESSED RZF AND BIR DOMAINS**

The assays of cell death described above indicate that the RZF acts as a negative regulator of the anti-apoptotic function of IAPs. One way in which the RZF, and possibly other IAP domains, may exert their regulatory influence is by altering the expression of genes, whose products function in the apoptotic pathway.

In order to determine whether the subcellular locations of expressed RZF and BIR domains are consistent with roles as nuclear regulatory factors, COS cells were transiently transfected with the following four constructs, and the expressed polypeptide was localized by immunofluorescent microscopy: (1) pcDNA3-6myc-XIAP, which encodes all 497 amino acids of SEQ ID NO: 4, (2) pcDNA3-6myc-m-XIAP, which encodes all 496 amino acids of mouse XIAP (SEQ ID NO: 10), (3) pcDNA3-6myc-mxiap-BIR, which encodes amino acids 1 to 341 of m-XIAP, and (4) pcDNA3-6myc-mxiap-RZF, which encodes amino acids 342-496 of murine XIAP. The cells were grown on multi-well tissue culture slides for 12 hours, and then fixed and permeabilized with methanol. The constructs used (here and in the cell death assays) were tagged with a human Myc epitope tag at the N-terminus. Therefore, a monoclonal anti-Myc antibody and a secondary goat anti-mouse antibody, which was conjugated to FITC, could be used to localize the expressed products in transiently transfected COS cells. Full-length XIAP and MIAP were located in the cytoplasm, with accentuated expression in the peri-nuclear zone. The same pattern of localization was observed when the cells expressed a construct encoding the RZF domain (but not the BIR domains). However, cells expressing the BIR domains (without the RZF) exhibited, primarily, nuclear staining. The protein expressed by the BIR domain construct appeared to be in various stages of transfer to the nucleus.

These observations are consistent with the fact that, as described below, XIAP is cleaved within T cells that are treated with anti-Fas antibodies (which are potent inducers of

apoptosis), and its N-terminal domain is translocated to the nucleus. As noted in Example 2, HIAP-2 appears to undergo a similar cleavage event.

**EXAMPLE 9: TESTING OF ANTISENSE OLIGONUCLEOTIDES:**

*1. Complete panel of adenovirus constructs.* The panel may consist of approximately four types of recombinant virus. A) Sense orientation viruses for each of the IAP or NAIP open reading frames: XIAP, HIAP-1, HIAP-2, and NAIP. These viruses are designed to massively overexpress the recombinant protein in infected cells. B) Antisense orientation viruses in which the viral promoter drives the synthesis of an mRNA of opposite polarity to the IAP mRNA, thereby shutting off host cell synthesis of the targeted protein coding region. XIAP, HIAP-1, HIAP-2, and NAIP "antisense" constructs required. C) Sub-domain expression viruses. These constructs express only a partial IAP protein in infected cells. Our results indicate that deletion of the zinc finger of XIAP renders the protein more potent in protecting cell against apoptotic triggers. This data also indicates that expression of the zinc finger alone will indicate apoptosis by functioning as a dominant-negative repressor of XIAP function. XIAP-ΔZF and XIAP-ΔBIR viruses required. D) Control viruses. Functional analysis of the IAPs requires suitable positive and negative controls for comparison. BCL-2 sense, BCL-2 antisense, p53 sense, and Lac Z (negative control) viruses may be utilized.

*2. Confirmation of recombinant adenovirus function.* Verification of the sense adenovirus function involves infection of tissue culture cells and determination of protein expression levels. We have performed western blot analysis of several of the recombinant adenoviruses, including NAIP, XIAP and XIAP-ΔRZF. The remaining viruses may be ready readily assessed for protein expression using the polyclonal IAP antibodies. Functional analysis of the antisense viruses may be done at the RNA level using either northern blots of total RNA harvested from infected tissue culture cells or ribonuclease protection assays. Western blot analysis of infected cells will be used to determine whether the expressed antisense RNA interferes with IAP expression in the host cell.

*3. Documentation that IAP overexpression results in increased drug resistance.* We have optimized cell death assays to allow high through-put of samples with minimal sample

variation. Testing of the sense IAP adenoviruses for their ability to alter drug sensitivity of breast and pancreatic adenocarcinoma cell lines may be accomplished as follows. Cancer cell lines are infected with the recombinant viruses, cultured for 5 days, then subdivided into 24 well plates. Triplicate cell receive increasing concentrations of the anti-cancer drug under investigation. Samples are harvested at 24, 48, and 72 hours post exposure, and assayed for the number of viable cells in the well. The dose response curve is then compared to uninfected and control virus (both positive and negative) infected cells. One may document a dramatic increase in the relative resistance of the cancer cell lines when infected with the sense viruses, confirming our hypothesis that overexpression of the IAP proteins contributes to the anti-apoptotic phenotype of cancer cells. Initial experiments utilize the chemotherapeutic drugs doxorubicin and adriamycin.

*4. Documentation that antisense IAP overexpression results in increased drug sensitivity.* Having confirmed that IAP overexpression renders cancer cell more resistant to chemotherapeutic drugs, one may examine whether the antisense adenoviruses render the same cells more sensitive. The effectiveness of antisense IAP viruses relative to antisense BCL-2 virus will also be assessed as a crucial milestone.

*5. Identification of antisense oligonucleotides.* Concomitant to the adenovirus work, we have designed a series of antisense oligonucleotides to various regions of each of the IAPs. A generally accepted model of how antisense oligonucleotides function proposes that the formation of RNA/DNA duplexes in the nucleus activates cellular RnaseH enzymes which then enzymatically degrade the mRNA component of the hybrid. Virtually any region of the mRNA can be targeted, and therefore choosing an appropriate sequence to target is somewhat empirical. Many factors, including secondary structure of the target mRNA and the binding affinity of the targeted sequence determine whether a particular oligonucleotide will be effective, necessitating several oligonucleotides for each IAP. Five oligonucleotides have been made for each IAP mRNA based on the available computer algorhythms for predicting binding affinities and mRNA secondary structures. These and other oligonucleotides may be tested for their ability to target their respective mRNAs for degradation using northern blot analysis.

6. *Optimization of oligonucleotides*. A secondary round of oligonucleotides may be made when effective target regions have been identified. These oligonucleotides target sequences in the immediate vicinity of the most active antisense oligonucleotides identified using methods such as those provided above. A second round of testing by northern blot analysis may be required.

7. *Testing antisense oligonucleotides in vitro*. Following successful identification and optimization of targeting oligonucleotides, one may test these in the tissue culture model system using the optimal cell lines such as those described in the cancer survey described herein. Experimental procedures may parallel those used in the recombinant antisense adenovirus work. Negative control oligonucleotides with miss-match sequences are used to establish base line or non-specific effects. Assisted transfection of the oligonucleotides using cationic lipid carriers may be compared to unassisted transfection. Confirmation of the effectiveness of specific antisense oligonucleotides prompts synthesis of oligonucleotides with modified phosphodiester linkages, such as phosphorothioate or methylimino substituted oligonucleotides. These may also be tested *in vitro*.

8. *Animal modeling of antisense oligonucleotide therapies*.

Animal modeling of the effectiveness of the antisense IAP approach is described here. Cell lines are routinely assessed for their tumorigenic potential in "nude" mice, a hairless strain of mouse that is immunocompromised (lacks a functional thymus), and thus extremely susceptible to developing tumors. In the nude mouse assay, cancer cells are grown in tissue culture and then injected under the skin at multiple sites. The frequency with which these cells give rise to palpable tumors within a defined period of time provides an index of the tumorigenic potential of the cell line in the absence of interference by a functional immune system. Preliminary assessment of an antisense IAP therapeutic involves injection of cancer cells infected with the recombinant adenoviruses (sense, antisense, and control viruses) under the skin, and the tumorigenic index compared to that of untreated cells. One may also use this model to assess the effectiveness of systemic administration of antisense oligonucleotides in increasing the efficacy of anti-cancer drugs in the nude mouse model. Phosphorothioate or methylimino substituted oligonucleotides will be assessed at this stage.

This type of antisense oligonucleotide has demonstrated enhanced cell permeability and slower clearance rates from the body in experimental animal models.

## EXAMPLE 10: ADDITIONAL APOPTOSIS ASSAYS

Specific examples of apoptosis assays are also provided in the following references.

Assays for apoptosis in lymphocytes are disclosed by: Li *et al.*, Science 268: 429-431, 1995; Gibellini *et al.*, Br. J. Haematol. 89: 24-33, 1995; Martin *et al.*, J. Immunol. 152: 330-342, 1994; Terai *et al.*, J. Clin Invest. 87: 1710-1715, 1991; Dhein *et al.*, Nature 373: 438-441, 1995; Katsikis *et al.*, J. Exp. Med. 1815: 2029-2036, 1995; Westendorp *et al.*, Nature 375: 497-500, 1995; DeRossi *et al.*, Virology 198: 234-244, 1994.

Assays for apoptosis in fibroblasts are disclosed by: Vossbeck *et al.*, Int. J. Cancer 61: 92-97, 1995; Goruppi *et al.*, Oncogene 9: 1537-1544, 1994; Fernandez *et al.*, Oncogene 9: 2009-2017, 1994; Harrington *et al.*, EMBO J., 13: 3286-3295, 1994; Itoh *et al.*, J. Biol. Chem. 268: 10932-10937, 1993.

Assays for apoptosis in neuronal cells are disclosed by: Melino *et al.*, Mol. Cell. Biol. 14: 6584-6596, 1994; Rosenbaum *et al.*, Ann. Neurol. 36: 864-870, 1994; Sato *et al.*, J. Neurobiol. 25: 1227-1234, 1994; Ferrari *et al.*, J. Neurosci. 1516: 2857-2866, 1995; Talley *et al.*, Mol. Cell. Biol. 15: 2359-2366, 1995; Walkinshaw *et al.*, J. Clin. Invest. 95: 2458-2464, 1995.

Assays for apoptosis in insect cells are disclosed by: Clem *et al.*, Science 254: 1388-1390, 1991; Crook *et al.*, J. Virol. 67: 2168-2174, 1993; Rabizadeh *et al.*, J. Neurochem. 61: 2318-2321, 1993; Birnbaum *et al.*, J. Virol. 68: 2521-2528, 1994; Clem *et al.*, Mol. Cell. Biol. 14: 5212-5222, 1994.

## EXAMPLE 11: CONSTRUCTION OF A TRANSGENIC ANIMAL

Characterization of IAP and NAIP genes provided information that necessary for generation IAP and NAIP transgenic animal models to be developed by homologous recombination (for knockouts) or transfection (for expression of IAP or NAIP fragments, antisense nucleic acids, or increased expression of wild-type or mutant IAPs or NAIP). Such a model may be a mammalian animal, *e.g.*, a mouse, and is useful for the identification of

cancer therapeutics alone or in combination with cancer inducing cells or agents, or when such mice are crossed with mice genetically predisposed to cancers.

The preferred transgenic animal overexpression in IAP or NAIP and has a predisposition to cancer. This mouse is particularly useful for the screening of potential cancer therapeutics.

## EXAMPLE 12: IAP OR NAIP PROTEIN EXPRESSION

IAP and NAIP genes and fragments thereof (*i.e.*, RZF fragments) may be expressed in both prokaryotic and eukaryotic cell types. If an IAP or NAIP fragment enhances apoptosis, it may be desirable to express that protein under control of an inducible promoter.

In general, IAPs and NAIP, and fragments thereof, may be produced by transforming a suitable host cell with all or part of the IAP-encoding or NAIP-encoding cDNA fragment that has been placed into a suitable expression vector.

Those skilled in the art of molecular biology will understand that a wide variety of expression systems may be used to produce the recombinant protein. The precise host cell used is not critical to the invention, although cancer cells are preferable. The IAP protein may be produced in a prokaryotic host (*e.g.*, *E. coli*) or in a eukaryotic host (*e.g.*, *S. cerevisiae*, insect cells such as Sf21 cells, or mammalian cells such as COS-1, NIH 3T3, or HeLa cells, or other highly proliferative cell types). These cells are publically available, for example, from the American Type Culture Collection, Rockville, MD; see also Ausubel *et al.*, *supra*). The method of transduction and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, *e.g.*, in Ausubel *et al.* (*supra*), and expression vehicles may be chosen from those provided, *e.g.*, in Cloning Vectors: A Laboratory Manual (P.H. Pouwels *et al.*, 1985, Supp. 1987).

Polypeptides of the invention, particularly short IAP fragments, can also be produced by chemical synthesis (*e.g.*, by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984 The Pierce Chemical Co., Rockford, IL). These general techniques of polypeptide expression and purification can also be used to produce and isolate useful IAP fragments or analogs, as described herein.

## EXAMPLE 13: ANTI-IAP AND ANTI-NAIP ANTIBODIES

In order to generate IAP-specific and NAIP-specific antibodies, an IAP or NAIP coding sequence (*e.g.*, amino acids 180-276) can be expressed as a C-terminal fusion with glutathione S-transferase (GST; Smith *et al.*, Gene 67: 31-40, 1988). The fusion protein can be purified on glutathione-Sepharose beads, eluted with glutathione, and cleaved with thrombin (at the engineered cleavage site), and purified to the degree required to successfully immunize rabbits. Primary immunizations can be carried out with Freund's complete adjuvant and subsequent immunizations performed with Freund's incomplete adjuvant. Antibody titres are monitored by Western blot and immunoprecipitation analyses using the thrombin-cleaved IAP fragment of the GST-IAP and GST-NAIP fusion proteins. Immune sera are affinity purified using CNBr-Sepharose-coupled IAP protein. Antiserum specificity is determined using a panel of unrelated GST proteins (including GSTp53, Rb, HPV-16 E6, and E6-AP) and GST-trypsin (which was generated by PCR using known sequences).

As an alternate or adjunct immunogen to GST fusion proteins, peptides corresponding to relatively unique hydrophilic regions of IAP or NAIP may be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides is similarly affinity purified on peptides conjugated to BSA, and specificity is tested by ELISA and Western blotting using peptide conjugates, and by Western blotting and immunoprecipitation using IAP or NAIP expressed as a GST fusion protein.

Alternatively, monoclonal antibodies may be prepared using the IAP or NAIP proteins described above and standard hybridoma technology (see, *e.g.*, Kohler *et al.*, Nature 256: 495, 1975; Kohler *et al.*, Eur. J. Immunol. 6: 511, 1976; Kohler *et al.*, Eur. J. Immunol. 6:292, 1976; Hammerling *et al.*, In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, New York, NY, 1981; Ausubel *et al.*, *supra*). Once produced, monoclonal antibodies are also tested for specific IAP or NAIP recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel *et al.*, *supra*).

Antibodies that specifically recognize IAPs or NAIP or fragments thereof, such as those described herein containing one or more BIR domains (but not a ring zinc finger domain), or that contain a ring zinc finger domain (but not a BIR domain) are considered useful in the invention. They may, for example, be used in an immunoassay to monitor IAP or NAIP expression levels or to determine the subcellular location of an IAP or NAIP (or

fragment thereof) produced by a mammal. Antibodies that inhibit the 26 kDa IAP cleavage product described herein (which contains at least one BIR domain) may be especially useful in inducing apoptosis in cells undergoing undesirable proliferation.

Preferably, antibodies of the invention are produced using IAP or NAIP sequence that does not reside within highly conserved regions, and that appears likely to be antigenic, as analyzed by criteria such as those provided by the Peptide structure program (Genetics Computer Group Sequence Analysis Package, Program Manual for the GCG Package, Version 7, 1991) using the algorithm of Jameson and Wolf (CABIOS 4: 181, 1988). Specifically, these regions, which are found between BIR1 and BIR2 of all IAPs, are: from amino acid 99 to amino acid 170 of HIAP-1, from amino acid 123 to amino acid 184 of HIAP-2, and from amino acid 116 to amino acid 133 of either XIAP or m-XIAP. These fragments can be generated by standard techniques, *e.g.*, by the PCR, and cloned into the pGEX expression vector (Ausubel *et al.*, *supra*). Fusion proteins are expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel *et al.* (*supra*). In order to minimize the potential for obtaining antisera that is non-specific, or exhibits low-affinity binding to IAP, two or three fusions are generated for each protein, and each fusion is injected into at least two rabbits. Antisera are raised by injections in series, preferably including at least three booster injections.

## EXAMPLE 14: IDENTIFICATION OF MOLECULES THAT MODULATE THE EXPRESSION OR BIOLOGICAL ACTIVITY OF AN IAP OR NAIP GENE

IAP and NAIP cDNAs facilitate the identification of molecules that decrease IAP or NAIP expression or otherwise enhance apoptosis normally blocked by these polypeptides. Such compounds are highly useful as, for example, chemotherapeutic agents to destroy a cancer cell, or to reduce the growth of a cancer cell, where the cancer cell is one, as is described herein, with an elevated level of an IAP or NAIP polypeptide.

In one approach, candidate molecules are added, in varying concentration, to the culture medium of cells expressing IAP or NAIP mRNA. IAP or NAIP expression is then measured, for example, by Northern blot analysis (Ausubel *et al.*, *supra*) using an IAP or NAIP cDNA, or cDNA fragment, as a hybridization probe. The level of IAP or NAIP expression in the presence of the candidate molecule is compared to the level of IAP or NAIP

expression in the absence of the candidate molecule, all other factors (*e.g.*, cell type and culture conditions) being equal.

The effect of candidate molecules on IAP- or NAIP-mediated apoptosis may, instead, be measured at the level of protein or the level of polypeptide fragments of IAP or NAIP polypeptides using the general approach described above with standard polypeptide detection techniques, such as Western blotting or immunoprecipitation with an IAP or NAIP-specific antibodies (for example, the antibodies described herein).

Compounds that modulate the level of a IAP or NAIP polypeptide may be purified, or substantially purified, or may be one component of a mixture of compounds such as an extract or supernatant obtained from cells (Ausubel *et al.*, *supra*). In an assay of a mixture of compounds, IAP or NAIP polypeptide expression is tested against progressively smaller subsets of the compound pool (*e.g.*, produced by standard purification techniques such as HPLC or FPLC) until a single compound or minimal number of effective compounds is demonstrated to modulate IAP or NAIP expression.

Compounds may also be screened for their ability to modulate the biological activity of an IAP or NAIP polypeptide by, for example, an ability to enhance IAP- or NAIP-mediated apoptosis. In this approach, the degree of apoptosis in the presence of a candidate compound is compared to the degree of apoptosis in its absence, under equivalent conditions. Again, the screen may begin with a pool of candidate compounds, from which one or more useful modulator compounds are isolated in a step-wise fashion. Apoptosis activity may be measured by any standard assay, for example, those described herein.

Another method for detecting compounds that modulate the expression or biological activity of an IAP or a NAIP polypeptide is to screen for compounds that interact physically with a given IAP polypeptide. These compounds may be detected by adapting two hybrid systems known in the art. These systems detect protein interactions using a transcriptional activation assay and are generally described by Gyuris *et al.* (Cell 75: 791-803, 1993) and Field *et al.* (Nature 340: 245-246, 1989), and are commercially available from Clontech (Palo Alto, CA). In addition, PCT Publication WO 95/28497 describes a two hybrid system in which proteins involved in apoptosis, by virtue of their interaction with BCL-2, are detected. A similar method may be used to identify proteins and other compounds that interact with IAP or NAIP polypeptides.

Compounds or molecules that function as modulators of IAP-mediated cell death may include peptide and non-peptide molecules such as those present in cell extracts, mammalian serum, or growth medium in which mammalian cells have been cultured. In addition, compounds previously known for their abilities to modulate apoptosis in cancer cells may be tested for an ability to modulate expression of an IAP molecule.

**TABLE 2**

OLIGONUCLEOTIDE PRIMERS FOR THE SPECIFIC RT-PCR AMPLIFICATION OF IAP GENES

| IAP Gene | Forward Primer (nucleotide position*) | Reverse Primer (nucleotide position*) | Size of Product (bp) |
|---|---|---|---|
| **h-XIAP** | p2415 (876-896) | p2449 (1291-1311) | 435 |
| **m-XIAP** | p2566 (458-478) | p2490 (994-1013) | 555 |
| **h-HIAP 1** | p2465 (827-847) | p2464 (1008-1038) | 211 |
| **m-HIAP 1** | p2687 (747-767) | p2684 (1177-1197) | 450 |
| **HIAP2** | p2595 (1562-1585) | p2578 (2339-2363) | 801& 618@ |
| **m-HIAP2** | p2693 (1751-1772) | p2734 (2078-2100) | 349 |

* Nucleotide position as determined from Figs. 1-4 for each IAP gene
&PCR product size of hiap2a
@ PCR product size of hiap2b

## EXAMPLE 15: ROLE OF IAPs IN HUMAN OVARIAN CANCER RESISTANCE TO CISPLATIN

Ovarian epithelial cancer cell apoptosis has been demonstrated to be involved in cisplatin-induced cell death (Havrilesky *et al.*, Obstet. Gynecol. 85: 1007-1010, 1995; Anthoney *et al.*, Cancer Res. 56: 1374-1381, 1996). The action of cisplatin is thought to involve the formation of inter and intra-strand DNA crosslinks (Sherman *et al.*, Science 230: 412-417, 1985) although the events leading to cell death after cisplatin treatment is unclear. If IAPs are indeed key elements in the regulation of apoptosis in ovarian cancer cells, one

would expect that down-regulation of this anti-apoptotic protein would result in cell death. To test this, cisplatin-sensitive human ovarian surface epithelial cells (OV2008) were infected with either adenoviral XIAP antisense, adenoviral HIAP-2 antisense, or the empty vector with LacZ (as control) for up to 60 hours, at which time changes in cell morphology, apoptotic cell number, cell viability, and total cell number were determined. The full length sense and antisense constructs of XIAP and HIAP-2 were prepared as briefly described hereafter. To construct the adenoviruses, the open reading frame for XIAP and HIAP-2 were PCR amplified with primers corresponding to the amino and carboxy terminus. These PCR products were cloned in the pCR2.1 vector (InvitroGen, Carlsbad, CA), and sequenced. The ORFs were then excised with EcoRI digestion, blunt ended with Klenow fragment, and ligated into SwaI digested pAdex1CAwt cosmid DNA. Packaging was performed with Promega (Madison, WI) cosmid packaging extracts and used to infect *E. coli*. Colonies were picked and screened for the presence of the insert in both the sense and antisense orientation relative to the chicken B-actin (CA) promoter. CsCl purified cosmid DNA was co-transfected with wild-type adenovirus DNA, which contains the terminal protein complexed to the ends of the DNA. Wild type adenovirus DNA was cut with NsiI such that only homologous recombinant with the cosmid DNA generated infectious adenovirus DNA. The final recombinant adenovirus contains a linear, double stranded genome of 44,820 bp plus the insert size (approximately 1,500 for XIAP, approximately 1,800 for HIAP-2).

Cisplatin-sensitive (OV2008) and cisplatin-resistant (C13) ovarian epithelial cancer cells were infected with adenovirus [multiplicity of infection (MOI) = 5 (1X); MOI = 10 (2X)] containing antisense XIAP or HIAP-2 cDNA, or vector (control) for 60 hours. Cells were then trypsinized and total cell number was determined with haemocytometry while cell viability was determined by the trypan blue dye exclusion test. XIAP antisense infection of OV2008 cells significantly increased the percentage of dead cells compared to control (vector, $p<0.001$), as determined by trypan blue exclusion tests (Fig. 25, top left panel). Although there appeared also to be a slight increase in percentage of dead cells with HIAP-2 antisense infection of OV2008 cells, it was not statistically significant (Fig. 25, top left panel; $p>0.05$). Infection of the cisplatin-resistant variant of OV2008 cells (C13) with antisense of XIAP but not of Hisp-2 also significantly, though to a lesser extent, decreased cell viability (Fig. 25, top right panel). The cell death induced in both OV2008 and C13 by XIAP

antisense was also accompanied by decreases in total cell number, with the effect of the antisense infection being more pronounced in the cisplatin-sensitive cells (Fig.25, bottom two panels).

In addition, 60 hours of adenoviral XIAP antisense infection of OV2008 decreased XIAP protein content and induced extensive cell detachment, as is shown in Fig. 26A (black arrows in left "b" photograph). Nuclear fragmentation (Fig. 26B, white arrows in photographs "b" and "d") and increased the number of apoptotic cells as well as the abundance of apoptotic bodies (Fig 26B: photographs "b" and "d" compared to "a" and "c") is also induced in OV2008 cells following 60 hours of infection with adenovirus XIAP antisense. For nuclear staining, cells were fixed in 4% formalin (in PBS, room temp., 10 min.) and washed in PBS. The washed cells were then resuspended in Hoechst staining solution (0.1 μg Hoechst 33248/ml PBS, 10 min.), washed again, and spotted onto slides for microscopy. Nuclear staining was observed and photographed using a Zeiss flourescence microscope. Cells with typical apoptotic nuclear morphology were identified and counted, suing randomly selected fields and numbered photographic slides to avoid bias during counting. Analysis of variance indicated that there was highly significant effects of the antisense on XIAP protein content ($p<0.001$; Fig. 26D and 26E) and apoptosis ($p<0.001$; Fig 26C). Indeed, infection of these cells with a higher titre of the adenoviral anti-sense (MOI=10 (2X)) further increased the number of cells undergoing apoptosis (Fig. 26C).

To study whether IAP expression is the target for the chemotherapeutic action of cisplatin, OV2008 cels were cultured in the absence and presence of cisplatin (10-30 μM) for 24 hours, apoptosis and XIAP and HIAP-2 expression were assessed morphologically and by Western analysis, respectively. Like adenoviral XIAP antisense infection, the presence of cisplatin induced morphologic feature of apoptosis in OV2008 cells, including decreased cell volume, chromatin condensation and nuclear fragmentation (Fig. 27A, left two photographs), and apoptotic low molecular weight DNA fragmentation (Fig. 27B), and was accompanied by decreased IAP expression (Figs. 28A and 28B). The increase of apoptotic cell number in response to cisplatin was also concentration-dependent and was significant (50% vs. 2%; $p<0.05$) even at a concentration of 10 μM cisplatin (Fig. 27C).

As shown in Figs. 28A and 28B, although both XIAP and HIAP-2 are present in the cisplatin-sensitive human ovarian surface epithelial cancer cell line OV2008 (protein sizes

55kDa and 68 kDa, respectively), their expression were down-regulated by cisplatin in a concentration-dependent manner. XIAP appearing more responsive to the anti-cancer agent. While XIAP protein content was decreased by almost 80% ($p<0.01$) in the presence 20 μM cisplatin. the decrease of HIAP-2 protein content was not suppressed by cisplatin (Figs. 28A and 28B).

The expression of XIAP and HIAP-2 in C13, the cisplatin-resistant variant of OV2008, was not suppressed by cisplatin (Figs. 28A and 28B), and no morphologic and biochemical changes characteristic of apoptosis could be detected (Figs. 27A and 27B). Although XIAP and HIAP-2 contents in C13 appeared to be higher in the presence of the anti-cancer agent, the differences were statistically non-significant ($p>0.05$). Time course experiments on IAP expression demonstrated that the suppression of XIAP and HIAP-2 protein levels in OV2008 by cisplatin was time-dependent; a significant decrease was observed between 12-24 hours of culture (Figs. 29A and 29B). Expression of XIAP and HIAP-2 in C13 cells was not influenced by cisplatin, irrespective of the duration of treatment.

To determine if the observed XIAP responses in OV2008 and C13 cells were specific to this pair of cell lines, the influence of cisplatin in vitro on XIAP and HIAP-2 protein content in another cisplatin-sensitive ovarian surface epithelial cancer cell line (A2780s) and its cisplatin-resistant variant (A2780cp) was studied (Figs. 30A and 30B). Interestingly, whereas HIAP-2 expression in both the sensitive and resistant cells was not significantly altered by the presence of the cisplatin (30 μM; Fig. 30B), XIAP protein content was decreased in A2780s (as in OV2008 cells) and not significantly altered in A2780cp (as in C13 cells) in the presence of the chemotherapeutic agent. Taken together, these data suggest that the apoptotic responsiveness of ovarian cancer cells to cisplatin may be related to the ability f the chemotherapeutic agent to down-regulate XIAP expression and that HIAP-2 may play a minor or no role in cisplatin-induced apoptosis.

To determine if XIAP expression is indeed the an important determinant in chemo-resistance in human ovarian surface epithelial cancer, the influence of cisplatin on XIAP protein content and apoptosis in OV2008 cells following adenoviral XIAP sense infection was investigated. While cisplatin reduced XIAP protein content in OV2008 cells infected with the empty vector (Figs. 31C and 31D, vector plus cisplatin), overexpression of the protein with adenoviral sense XIAP cDNA 48 hrs prior to treatment with the

chemotherapeutic agent *in vitro* attenuated the cisplatin effects not only on XIAP protein expression (Figs. 31C and 31D) but also apoptotic nuclear fragmentation (Fig. 31A, "d" compared to "c") and number of apoptotic cells (Fig. 31B), suggesting that XIAP may be an important element in human ovarian epithelial cancer chemoresistance.

The *in vitro* studies with ovarian epithelial cancer cell lines strongly suggest an important role of IAPs, particularly of XIAP, in the control of apoptosis and tumor progression in human ovarian cancer. To determine if indeed IAPs are expressed in ovarian carcinoma and thus of clinical relevance, XIAP and HIAP-2 were immunlocalized in human ovarian surface epithelial tumors obtained as pathological samples from patients during surgical debulking, using polyclonal antibodies (rabbit polyclonal anti-XIAP and HIAP-2 antibodies were prepared by immunization with human XIAP and HIAP-2 GST fusion protein) against human XIAP and HIAP-2, respectively (Figs. 32C and 32D, respectively). In addition, *in situ* TUNEL (described in Gavrieli *et al.*, J. Cell. Biol. 119: 493-501, 1992) and immunohistochemistry for PCNA (proliferating cell nuclear antigen: an auxiliary protein of DNA polymerase α highly expressed as the G1/S interphase) were performed to examine if and how the expression of these IAPs relates to epithelial cell apoptosis and /or proliferation. Ovarian epithelial tumors exhibited considerable cellular heterogeneity (Fig. 32A) and PCNA positive cells were evident throughout the nucleus in the tumor section (Fig. 32B). In general, most of the cells were TUNEL negative (Fig. 32A), and the expression of XIAP and HIAP-2 was highly correlated to the proliferative state of the cells and inversely related to epithelial cell death. XIAP and HIAP-2 immunoreactivity (Figs. 32C and 32D, respectively) specifically localized in the cytoplasm or the perinuclear region was highest in proliferatively active cells (PCNA positive) and was low or absent in apoptotic cells (TUNEL positive) occasionally found in the tumor specimens.

## EXAMPLE 16: ADDITIONAL CANCER THERAPIES

Given the increased proliferation rate of cancer cells, it is preferable in anti-cancer therapeutic regimes to initiate treatment with an anti-cancer agent that will successfully inhibit the growth of the particular cancer of interest. One method to detect such an agent is to excise proliferative cells from the cancer of interest, and determine the level of expression

and/or level of biological activity of each individual IAP or NAIP polypeptide, and compare these levels to the levels of these polypeptide in a similar cell type from an unaffected individual. For example, if an human female individual has breast cancer (or a neoplasm suspected of being cancerous), cells from the cancer collected, for example, during a biopsy of the cancer, can be isolated and, if necessary, propagated in culture. The cells can then be analyzed for level of expression and/or level of biological activity of all of the IAP and NAIP polypeptides in the cell. The expression levels and/or biological activity levels of these polypeptides from the proliferating cells can be compared to the levels of expression and/or biological activity of these polypeptides from normal, healthy cells from a human female individual. Preferably, the comparison is made between on affected (*i.e.*, abnormally proliferating) and healthy cells of the same individual (*e.g.*, cells taken from healthy breast tissue from the individual being tested. The level of expression and/or biological activity of each polypeptide in the affected cells is compared to its counterpart in the healthy cells. Any increase in any (or all) of the IAP or NAIP polypeptides is detected. The cancer is then treated with a compound that decreases expression level or biological activity level of each particular elevated IAP or NAIP polypeptide. Methods for identifying such compounds are described above (see, *e.g.*, Example 14).

It will be understood that the individual undergoing such analysis and treatment may have already received treatment with an anti-cancer therapeutic agent. It will also be understood that, in addition to targeting the levels of expression and/or biological activities of IAP and NAIP polypeptides, the anti-cancer compounds may also target these levels for other apoptosis-inhibiting polypeptides, such as BCL-2. For example, an individual with breast cancer whose proliferating cells have an increased level of XIAP compared to the level of XIAP in healthy breast cells may be treated with a compound (*e.g.*, cisplatin) plus a compound that targets another IAP polypeptide, or that targets an NAIP polypeptide or a non-related apoptosis-inhibiting polypeptide, such as BCL-2).

One rapid method to determine expression levels of IAP and NAIP polypeptides is an ELISA assay using antibodies that specifically binds each of these polypeptides. Other methods include quantitative PCR and the various apoptosis assays described herein.

**EXAMPLE 17: ASSIGNMENT OF XIAP, HIAP-1, AND HIAP-2 TO CHROMOSOMES XQ25 AND 11Q22-23 BY FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH)**

Fluorescence *in situ* hybridization (FISH) was used to identify the chromosomal location of XIAP, HIAP-1 and HIAP-2.

A total of 101 metaphase spreads were examined with the XIAP probe, as described above. Symmetrical fluorescent signals on either one or both homologs of chromosome Xq25 were observed in 74% of the cells analyzed. Following staining with HIAP-1 and HIAP-2 probes, 56 cells were analyzed and doublet signals in the region 11q22-23 were observed in 83% of cells examined. The XIAP gene was mapped to Xq25 while the HIAP-1 and HIAP-2 genes were mapped at the border of 11q22 and 11q23 bands.

These experiments confirmed the location of the XIAP gene on chromosome Xq25. No highly consistent chromosomal abnormalities involving band Xq25 have been reported so far in any malignancies. However, deletions within this region are associated with a number of immune system defects including X-linked lymphoproliferative disease (Wu *et al.*, Genomics 17:163-170, 1993).

Cytogenetic abnormalities of band 11q23 have been identified in more than 50% of infant leukemias regardless of the phenotype (Martinez-Climet *et al.*, Leukaemia 9: 1299-1304, 1995). Rearrangements of the MLL Gene (mixed lineage leukemia or myeloid lymphoid leukemia; Ziemin-van der Poel *et al.*, Proc. Natl. Acad. Sci. USA 88: 10735-10739, 1991) have been detected in 80% of cases with 11q23 translocation, however patients whose rearrangements clearly involved regions other than the MLL gene were also reported (Kobayashi *et al.*, Blood 82: 547-551, 1993). Thus, the IAP genes may follow the BCL-2 paradigm, and would therefore play an important role in cancer transformation.

Incorporation by Reference

The following documents and all the references referred to herein are incorporated by reference: U.S.S.N. 08/511,485, filed August 4, 1995; U.S.S.N. 08/576,956, filed December 22, 1995; PCT/IB96/01022, filed August 5, 1996; U.S.S.N. 60/017,354, filed April 26, 1996; U.S.S.N. 60/030,931, filed November 15, 1996; U.S.S.N. 60/030,590, filed November 14, 1996; U.S.P.N. 5,576,208, issued November 19, 1996; and PCT Application No.

IB97/00142, filed January 17, 1997 claiming priority from UK 9601108.5, filed January 19, 1996.

Other Embodiments

In other embodiments, the invention includes use of any protein which is substantially identical to a mammalian IAP polypeptides (Figs. 1-6; SEQ ID Nos: 3-14); such homologs include other substantially pure naturally-occurring mammalian IAP proteins as well as allelic variants; natural mutants; induced mutants; DNA sequences which encode proteins and also hybridize to the IAP DNA sequences of Figs. 1-6 (SEQ ID NOS: 3-14) under high stringency conditions or, less preferably, under low stringency conditions (*e.g.*, washing at 2X SSC at 40°C with a probe length of at least 40 nucleotides); and proteins specifically bound by antisera directed to a IAP polypeptide. The term also includes chimeric polypeptides that include a IAP portion.

The invention further includes use of analogs of any naturally-occurring IAP polypeptide. Analogs can differ from the naturally-occurring IAP protein by amino acid sequence differences, by post-translational modifications, or by both. Analogs of the invention will generally exhibit at least 85%, more preferably 90%, and most preferably 95% or even 99% identity with all or part of a naturally occurring IAP amino acid sequence. The length of sequence comparison is at least 15 amino acid residues, preferably at least 25 amino acid residues, and more preferably more than 35 amino acid residues. Modifications include *in vivo* and *in vitro* chemical derivatization of polypeptides, *e.g.*, acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring IAP polypeptide by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual, 2nd ed., CSH Press, 1989, or Ausubel *et al.*, *supra*). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, *e.g.*, D-amino acids or nonnaturally occurring or synthetic amino acids, *e.g.*, B or y amino acids. In addition to full-length polypeptides, the invention also includes IAP

polypeptide fragments. As used herein, the term "fragment," means at least 20 contiguous amino acids, preferably at least 30 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least 60 to 80 or more contiguous amino acids. Fragments of IAP polypeptides can be generated by methods known to those skilled in the art or may result from normal protein processing (*e.g.*, removal of amino acids from the nascent polypeptide that are not required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

Preferable fragments or analogs used according to the methods of the invention are those which facilitate specific detection of an IAP nucleic acid or amino acid sequence in a sample to be diagnosed. Particularly useful IAP fragments for this purpose include, without limitation, the amino acid fragments shown in Table 2.

The methods of the invention may use antibodies prepared by a variety of methods. For example, the IAP or NAIP polypeptide, or antigenic fragments thereof, can be administered to an animal in order to induce the production of polyclonal antibodies. Alternatively, antibodies used as described herein may be monoclonal antibodies, which are prepared using hybridoma technology (see, *e.g.*, Kohler *et al.*, Nature 256: 495-497, 1975; Kohler *et al.*, Eur. J. Immunol. 6: 511-519, 1976; Kohler *et al.*, Eur. J. Immunol. 6: 292-295, 1976; Hammerling *et al.*, In Monoclonal Antibodies and T Cell Hybridomas, Elsevier, NY, 1981; Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988). The invention features use of antibodies that specifically bind human or murine IAP or NAIP polypeptides, or fragments thereof. In particular the invention features "neutralizing" antibodies. By "neutralizing" antibodies is meant antibodies that interfere with any of the biological activities of IAP or NAIP polypeptides, particularly the ability of IAPs to inhibit apoptosis. The neutralizing antibody may reduce the ability of IAP polypeptides to inhibit polypeptides by, preferably 50%, more preferably by 70%, and most preferably by 90% or more. Any standard assay of apoptosis, including those described herein, by those incorporated by reference and those in the art, may be used to assess neutralizing antibodies.

In addition to intact monoclonal and polyclonal anti-IAP antibodies, the invention features use of various genetically engineered antibodies, humanized antibodies, and antibody fragments, including F(ab')2, Fab', Fab, Fv and sFv fragments. Antibodies can be

humanized by methods known in the art, *e.g.*, monoclonal antibodies with a desired binding specificity can be commercially humanized (Scotgene, Scotland; Oxford Molecular, Palo Alto, CA). Fully human antibodies, such as those expressed in transgenic animals, are also features of the invention (Green *et al.*, Nature Genetics 7:13-21, 1994).

Ladner (U.S. Patent Nos. 4,946,778 and 4,704,692) describes methods for preparing single polypeptide chain antibodies. Ward *et al.* (Nature 341: 544-546, 1989) describe the preparation of heavy chain variable domains, which they term "single domain antibodies," which have high antigen-binding affinities. McCafferty *et al.* (Nature 348: 552-554, 1990) show that complete antibody V domains can be displayed on the surface of fd bacteriophage, that the phage bind specifically to antigen, and that rare phage (one in a million) can be isolated after affinity chromatography. Boss *et al.* (U.S. Patent No. 4,816,397) describe various methods for producing immunoglobulins, and immunologically functional fragments thereof, which include at least the variable domains of the heavy and light chain in a single host cell. Cabilly *et al.* (U.S. Patent No. 4,816,567) describe methods for preparing chimeric antibodies.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: University of Ottawa

(ii) TITLE OF THE INVENTION: DETECTION AND MODULATION OF IAPS AND NAIP FOR THE DIAGNOSIS AND TREATMENT OF PROLIFERATIVE DISEASE

(iii) NUMBER OF SEQUENCES: 17

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 176 Federal Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02110

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/---
(B) FILING DATE: 13-FEB-1998
(C) CLASSIFICATION:

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(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

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(B) REGISTRATION NUMBER: 39,109

(C) REFERENCE/DOCKET NUMBER: 07891/009WO2

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(A) TELEPHONE: 617-428-0200

(B) TELEFAX: 617-428-7045

(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(D)OTHER INFORMATION: Xaa at positions 2, 3, 4, 5, 6, 7, 9, 10, 11, 17, 18, 19, 20, 21, 23, 25, 30, 31, 32, 34, 35, 38, 39, 40, 41, 42, and 45 may be any amino acid. Xaa at position 8 is Glu or Asp. Xaa at positions 14 & 22 is Val or Ile.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Lys Xaa Cys Met
 1               5                  10                  15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Phe Xaa Pro Cys Gly His Xaa Xaa Xaa
            20                  25                  30
Cys Xaa Xaa Cys Ala Xaa Xaa Xaa Xaa Xaa Cys Pro Xaa Cys
        35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(v) FRAGMENT TYPE: internal
(ix) FEATURE:
(D) OTHER INFORMATION: Xaa at positions 1, 2, 3, 6, 9, 10, 14, 15, 18, 19, 20, 21, 24, 30, 32, 33, 35, 37, 40, 42, 43, 44, 45, 46, 47, 49, 50, 51, 53, 54, 55, 56, 57, 59, 60, 61, 62, 64 and 66 may be any amino acid. Xaa at positions 13, 16 and 17 may be any amino acid or may be absent.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Xaa Xaa Arg Leu Xaa Thr Phe Xaa Xaa Trp Pro Xaa Xaa Xaa Xaa
1 5 10 15
Xaa Xaa Xaa Xaa Xaa Leu Ala Xaa Ala Gly Phe Tyr Tyr Xaa Gly Xaa
20 25 30
Xaa Asp Xaa Val Xaa Cys Phe Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Trp
35 40 45
Xaa Xaa Xaa Asp Xaa Xaa Xaa Xaa Xaa His Xaa Xaa Xaa Xaa Pro Xaa
50 55 60
Cys Xaa Phe Val
65

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5232 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAAAAGGTGG ACAAGTCCTA TTTTCAAGAG AAGATGACTT TTAACAGTTT TGAAGGATCT 60
AAAACTTGTG TACCTGCAGA CATCAATAAG GAAGAAGAAT TTGTAGAAGA GTTTAATAGA 120
TTAAAAACTT TTGCTAATTT TCCAAGTGGT AGTCCTGTTT CAGCATCAAC ACTGGCACGA 180
GCAGGGTTTC TTTATACTGG TGAAGGAGAT ACCGTGCGGT GCTTTAGTTG TCATGCAGCT 240
GTAGATAGAT GGCAATATGG AGACTCAGCA GTTGGAAGAC ACAGGAAAGT ATCCCCAAAT 300
TGCAGATTTA TCAACGGCTT TTATCTTGAA AATAGTGCCA CGCAGTCTAC AAATTCTGGT 360
ATCCAGAATG GTCAGTACAA AGTTGAAAAC TATCTGGGAA GCAGAGATCA TTTTGCCTTA 420
GACAGGCCAT CTGAGACACA TGCAGACTAT CTTTTGAGAA CTGGGCAGGT TGTAGATATA 480
TCAGACACCA TATACCCGAG GAACCCTGCC ATGTATAGTG AAGAAGCTAG ATTAAAGTCC 540
TTTCAGAACT GGCCAGACTA TGCTCACCTA ACCCCAAGAG AGTTAGCAAG TGCTGGACTC 600
TACTACACAG GTATTGGTGA CCAAGTGCAG TGCTTTTGTT GTGGTGGAAA ACTGAAAAAT 660
TGGGAACCTT GTGATCGTGC CTGGTCAGAA CACAGGCGAC ACTTTCCTAA TTGCTTCTTT 720
GTTTTGGGCC GGAATCTTAA TATTCGAAGT GAATCTGATG CTGTGAGTTC TGATAGGAAT 780
TTCCCAAATT CAACAAATCT TCCAAGAAAT CCATCCATGG CAGATTATGA AGCACGGATC 840
TTTACTTTTG GGACATGGAT ATACTCAGTT AACAAGGAGC AGCTTGCAAG AGCTGGATTT 900
TATGCTTTAG GTGAAGGTGA TAAAGTAAAG TGCTTTCACT GTGGAGGAGG GCTAACTGAT 960
TGGAAGCCCA GTGAAGACCC TTGGGAACAA CATGCTAAAT GGTATCCAGG GTGCAAATAT 1020
CTGTTAGAAC AGAAGGGACA AGAATATATA AACAATATTC ATTTAACTCA TTCACTTGAG 1080
GAGTGTCTGG TAAGAACTAC TGAGAAAACA CCATCACTAA CTAGAAGAAT TGATGATACC 1140
ATCTTCCAAA ATCCTATGGT ACAAGAAGCT ATACGAATGG GGTTCAGTTT CAAGGACATT 1200
AAGAAAATAA TGGAGGAAAA AATTCAGATA TCTGGGAGCA ACTATAAATC ACTTGAGGTT 1260
CTGGTTGCAG ATCTAGTGAA TGCTCAGAAA GACAGTATGC AAGATGAGTC AAGTCAGACT 1320
TCATTACAGA AAGAGATTAG TACTGAAGAG CAGCTAAGGC GCCTGCAAGA GGAGAAGCTT 1380
TGCAAAATCT GTATGGATAG AAATATTGCT ATCGTTTTTG TTCCTTGTGG ACATCTAGTC 1440
ACTTGTAAAC AATGTGCTGA AGCAGTTGAC AAGTGTCCCA TGTGCTACAC AGTCATTACT 1500
TTCAAGCAAA AAATTTTTAT GTCTTAATCT AACTCTATAG TAGGCATGTT ATGTTGTTCT 1560
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ATTCATAGTA TACTGATTTA ATTTCTAAGT GTAAGTGAAT TAATCATCTG GATTTTTTAT 1860
TCTTTTCAGA TAGGCTTAAC AAATGGAGCT TTCTGTATAT AAATGTGGAG ATTAGAGTTA 1920
ATCTCCCCAA TCACATAATT TGTTTTGTGT GAAAAAGGAA TAAATTGTTC CATGCTGGTG 1980

GAAAGATAGA GATTGTTTTT AGAGGTTGGT TGTTGTGTTT TAGGATTCTG TCCATTTTCT 2040
TTTAAAGTTA TAAACACGTA CTTGTGCGAA TTATTTTTTT AAAGTGATTT GCCATTTTTG 2100
AAAGCGTATT TAATGATAGA ATACTATCGA GCCAACATGT ACTGACATGG AAAGATGTCA 2160
AAGATATGTT AAGTGTAAAA TGCAAGTGGC AAAACACTAT GTATAGTCTG AGCCAGATCA 2220
AAGTATGTAT GTTTTTAATA TGCATAGAAC AAAAGATTTG GAAAGATATA CACCAAACTG 2280
TTAAATGTGG TTTCTCTTCG GGGAGGGGGG GATTGGGGGA GGGGCCCCAG AGGGGTTTTA 2340
TAGGGGCCTT TTCACTTTCT ACTTTTTTCA TTTTGTTCTG TTCGAATTTT TTATAAGTAT 2400
GTATTACTTT TGTAATCAGA ATTTTTAGAA AGTATTTTGC TGATTTAAAG GCTTAGGCAT 2460
GTTCAAACGC CTGCAAAACT ACTTATCACT CAGCTTTAGT TTTTCTAATC CAAGAAGGCA 2520
GGGCAGTTAA CCTTTTTGGT GCCAATGTGA AATGTAAATG ATTTTATGTT TTTCCTGCTT 2580
TGTGGATGAA AAATATTTCT GAGTGGTAGT TTTTTGACAG GTAGACCATG TCTTATCTTG 2640
TTTCAAAATA AGTATTTCTG ATTTTGTAAA ATGAAATATA AAATATGTCT CAGATCTTCC 2700
AATTAATTAG TAAGGATTCA TCCTTAATCC TTGCTAGTTT AAGCCTGCCT AAGTCACTTT 2760
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TATCATCTTG TATCTTAAAG TTTCATGTGA GTTTTTACCG TTAGGATGAT TAAGATGTAT 2940
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GTAGATACTT CTGAAATAAA TGTTCTCTCA AGATCCTTAA AACCTCTTGG AAATTATAAA 3060
AATATTGGCA AGAAAAGAAG AATAGTTGTT TAAATATTTT TTAAAAAACA CTTGAATAAG 3120
AATCAGTAGG GTATAAACTA GAAGTTTAAA AATGCCTCAT AGAACGTCCA GGGTTTACAT 3180
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AGTTTGAGAG TAAAACTGTA AAAAATTATA TTTTTGTTGT ACTTTCTAAG AGAAAGAGTA 3300
TTGTTATGTT CTCCTAACTT CTGTTGATTA CTACTTTAAG TGATATTCAT TTAAAACATT 3360
GCAAATTTAT TTTATTTATT TAATTTTCTT TTTGAGATGG AGTCTTGCTT GTCACCCAGG 3420
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TCTCGTGCCT CAGCTTCCTG AGTAGCTGGA ATTACAGGCA GGTGCCACCA TGCCCGACTA 3540
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TCCTGACCTC AAGAGATCCA CTCGCCTTGC CCTCCCAAAG TGCTGGGATT ACAGGCTTGA 3660
GCCACCACGC CCGGCTAAAA CATTGCAAAT TTAAATGAGA GTTTTAAAAA TTAAATAATG 3720
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AAACTTGTAT GTTTAGAGTT AAGCAAGACT TTTTTTCTTC CTCTCCATGA GTTGTGAAAT 4080
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GAGTTCTCAG TGTCTACATG TAGACTATTC CTTTTCTGTA TAAAGTTCAC TCTAGGATTT 4380
CAAGTCACCA CTTATTTTAC ATTTTAGTCA TGCAAAGATT CAAGTAGTTT TGCAATAAGT 4440
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AXXGGGTGTA GTGAGTGTAT ATAATGTGAT TTGGCCCTGT GTATTATGAT ATTTTGTTAT 4680
TTTTGTTGTT ATATTATTTA CATTTCAGTA GTTGTTTTTT GTGTTTCCAT TTTAGGGGAT 4740
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TACCCTTTAA ACCCGAATCA TTGTTTTATT TCCTGATTAC ACAGGTGTTG AATGGGGAAA 4860
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ATGAAATAAA ATGGGGCTGG GCTCAGTGGC TCACGCCTGT AATCCCAGCA CTTTGGGAGG 4980
CTGAGGCAGG TGGATCACGA GGTCAGGAGA TCGAGACCAT CCTGGCTAAC ACGGTGAAAC 5040
CCCGTCTCTA CTAAAAAACA GAAAATTAGC CGGGCGTGGT GGCGGGCGCC TGTAGTCCCA 5100
GCTACTCGGG AGGCTGAGGC AGGAGAATGG TGTGAACCCG GGAGGCAGAG CTTGCAGTGA 5160
GCCGAGATCT CGCCACTGCA CTCCAGCCTG GGCAACAGAG CAAGACTCTG TCTCAAAAAA 5220
AAAAAAAAAA AG 5232

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 497 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Phe Asn Ser Phe Glu Gly Ser Lys Thr Cys Val Pro Ala Asp
1 5 10 15
Ile Asn Lys Glu Glu Glu Phe Val Glu Glu Phe Asn Arg Leu Lys Thr
20 25 30
Phe Ala Asn Phe Pro Ser Gly Ser Pro Val Ser Ala Ser Thr Leu Ala
35 40 45

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Arg Ala Gly Phe Leu Tyr Thr Gly Glu Gly Asp Thr Val Arg Cys Phe
    50                  55                  60
Ser Cys His Ala Ala Val Asp Arg Trp Gln Tyr Gly Asp Ser Ala Val
65                  70                  75                  80
Gly Arg His Arg Lys Val Ser Pro Asn Cys Arg Phe Ile Asn Gly Phe
                85                  90                  95
Tyr Leu Glu Asn Ser Ala Thr Gln Ser Thr Asn Ser Gly Ile Gln Asn
            100                 105                 110
Gly Gln Tyr Lys Val Glu Asn Tyr Leu Gly Ser Arg Asp His Phe Ala
        115                 120                 125
Leu Asp Arg Pro Ser Glu Thr His Ala Asp Tyr Leu Leu Arg Thr Gly
    130                 135                 140
Gln Val Val Asp Ile Ser Asp Thr Ile Tyr Pro Arg Asn Pro Ala Met
145                 150                 155                 160
Tyr Cys Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr
                165                 170                 175
Ala His Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr
            180                 185                 190
Gly Ile Gly Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys
        195                 200                 205
Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe
    210                 215                 220
Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Leu Asn Ile Arg Ser Glu
225                 230                 235                 240
Ser Asp Ala Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Leu
                245                 250                 255
Pro Arg Asn Pro Ser Met Ala Asp Tyr Glu Ala Arg Ile Phe Thr Phe
            260                 265                 270
Gly Thr Trp Ile Tyr Ser Val Asn Lys Glu Gln Leu Ala Arg Ala Gly
        275                 280                 285
Phe Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly
    290                 295                 300
Gly Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Glu Gln His
305                 310                 315                 320
Ala Lys Trp Tyr Pro Gly Cys Lys Tyr Leu Leu Glu Gln Lys Gly Gln
                325                 330                 335
Glu Tyr Ile Asn Asn Ile His Leu Thr His Ser Leu Glu Glu Cys Leu
```

340 345 350

Val Arg Thr Thr Glu Lys Thr Pro Ser Leu Thr Arg Arg Ile Asp Asp

355 360 365

Thr Ile Phe Gln Asn Pro Met Val Gln Glu Ala Ile Arg Met Gly Phe

370 375 380

Ser Phe Lys Asp Ile Lys Lys Ile Met Glu Glu Lys Ile Gln Ile Ser

385 390 395 400

Gly Ser Asn Tyr Lys Ser Leu Glu Val Leu Val Ala Asp Leu Val Asn

405 410 415

Ala Gln Lys Asp Ser Met Gln Asp Glu Ser Ser Gln Thr Ser Leu Gln

420 425 430

Lys Glu Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys

435 440 445

Leu Cys Lys Ile Cys Met Asp Arg Asn Ile Ala Ile Val Phe Val Pro

450 455 460

Cys Gly His Leu Val Thr Cys Lys Gln Cys Ala Glu Ala Val Asp Lys

465 470 475 480

Cys Pro Met Cys Tyr Thr Val Ile Thr Phe Lys Gln Lys Ile Phe Met

485 490 495

Ser

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6669 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTGCTCTGTC ACCCAGTTTG GAGTGCAGTT ATGCAGTCTC ACACTGCAAG CTCTGCCTCA 60

TGGGCTCAAG TGAACCTCCT GCCTCAGCCT CTCAAGTAGC TGGGACCACA GGCAGGTGCC 120

ACCATGTCTG GCTAATTTTT GAGTTTCTTT GTAGAGATGG TGTTTTGCCA AGTCACCCAG 180

TTTGAGGCTG GTCTCAAACA CCTGGGCTCA AGCAATCCAT CTACCTCAGC CTCCCAAAGT 240
GCTGGGATTA CAGGAGTGAG CCATGGCATG AGGCCTTGTG GGGTGTCTCT TTTAAATGAA 300
AGCATACTCT GTTTACGTAT TTGATATGAA GGAATATCCT TCCTTTCCAC AAAGACAAAA 360
ATTATCCTAT TTTTCTCAAA ACATATGTCC TTTTTCTCTA CTTTTCATTT TTGTTACTTT 420
TGATGGACAC ATGTGTTACA TTGATTTCAC TTTCTCATAA TTCTGCTGTA AGAAAAACAA 480
TAGTGCCAGT TCAATGACAA ATAGCAACAG TCTGTTATTG CTAGACTGTT ACTGTTAGTG 540
GAGACTACCA GAACAGTCAG TCCCAGTGTC AGGGAATCAA AGAGAACATG TTCCCTCTCT 600
AAAGGGCACA GCTGCTGCTC AGCTTTAGCT GATTGCTGCC CTGCAGGACT ATAGGCCCAG 660
TGTTGCTAGA TCTTTTGATG TTTCAAGAGA AGCTTGGAAT CTAGAATGTG ATGGGAAGTC 720
TCTTACATTT AAACATGTTG GCAATTAATG GTAAGATTTA AAAATACTGT GGTCCAAGAA 780
AAAAATGGAT TTGGAAACTG GATTAAATTC AAATGAGGCA TGCAGATTAA TCTACAGCAT 840
GGTACAATGT GAATTTTCTG GTTTCTTTAA TTGCACTGTA ATTAGGTAAG ATGTTAGCTT 900
TGGGGAAGCT AAGTGCAGAG TATGCAGAAA CTATTATTTT TGTAAGTTTT CTCTAAGTAT 960
AAATAAATTT CAAAATAAAA ATAAAAACTT AGTAAAGAAC TATAATGCAA TTCTATGTAA 1020
GCCAAACATA ATATGTCTTC CAGTTTGAAA CCTCTGGGTT TTATTTTATT TTATTTTATT 1080
TTTGAGACAG AGTCTTGCTG TGTCACCCAG GCTGGAGTGT AGTGGCACTA TTTCGGCCCA 1140
CTGCAACCTC CACCTCCCAG GCTCAAATGA TTCTCCTGCC TCAGCCTCCG GAGTAGCTGG 1200
GATTACAGGC GCGTACCACC ACACCCAGCT AATTTTTGTA TTTTTAGTAG AGATGGGGTT 1260
TCACCATTTT GGCCAGGCTG GTTTTGAACT CCTGACCTCA AGTGATCCAC TTGTCTTGGC 1320
CTCCCAAAAT GCTGGGATTA CAGGCGTGAG CCACTGCACC AGGCAGAGGC CTCTGTTTTT 1380
TATCTCTTTT TGGCCTCTAC AGTGCCTAGT AAAGCACCTG ATACATGGTA AACGATCAGT 1440
AATTACTAGT ACTCTATTTT GGAGAAAATG ATTTTTTAAA AAGTCATTGT GTTCCATCCA 1500
TGAGTCGTTT GAGTTTTAAA ACTGTCTTTT TGTTTGTTTT TGAACAGGTT TACAAAGGAG 1560
GAAAACGACT TCTTCTAGAT TTTTTTTTCA GTTTCTTCTA TAAATCAAAA CATCTCAAAA 1620
TGGAGACCTA AAATCCTTAA AGGGACTTAG TCTAATCTCG GGAGGTAGTT TTGTGCATGG 1680
GTAAACAAAT TAAGTATTAA CTGGTGTTTT ACTATCCAAA GAATGCTAAT TTTATAAACA 1740
TGATCGAGTT ATATAAGGTA TACCATAATG AGTTTGATTT TGAATTTGAT TTGTGGAAAT 1800
AAAGGAAAAG TGATTCTAGC TGGGGCATAT TGTTAAAGCA TTTTTTTCAG AGTTGGCCAG 1860
GCAGTCTCCT ACTGGCACAT TCTCCCATTA TGTAGAATAG AAATAGTACC TGTGTTTGGG 1920
AAAGATTTTA AAATGAGTGA CAGTTATTTG GAACAAAGAG CTAATAATCA ATCCACTGCA 1980
AATTAAAGAA ACATGCAGAT GAAAGTTTTG ACACATTAAA ATACTTCTAC AGTGACAAAG 2040
AAAAATCAAG AACAAAGCTT TTTGATATGT GCAACAAATT TAGAGGAAGT AAAAAGATAA 2100
ATGTGATGAT TGGTCAAGAA ATTATCCAGT TATTTACAAG GCCACTGATA TTTTAAACGT 2160
CCAAAAGTTT GTTTAAATGG GCTGTTACCG CTGAGAATGA TGAGGATGAG AATGATGGTT 2220
GAAGGTTACA TTTTAGGAAA TGAAGAAACT TAGAAAATTA ATATAAAGAC AGTGATGAAT 2280
ACAAAGAAGA TTTTTATAAC AATGTGTAAA ATTTTTGGCC AGGGAAAGGA ATATTGAAGT 2340
TAGATACAAT TACTTACCTT TGAGGGAAAT AATTGTTGGT AATGAGATGT GATGTTTCTC 2400

CTGCCACCTG GAAACAAAGC ATTGAAGTCT GCAGTTGAAA AGCCCAACGT CTGTGAGATC 2460
CAGGAAACCA TGCTTGCAAA CCACTGGTAA AAAAAAAAAA AAAAAAAAAA AAAGCCACAG 2520
TGACTTGCTT ATTGGTCATT GCTAGTATTA TCGACTCAGA ACCTCTTTAC TAATGGCTAG 2580
TAAATCATAA TTGAGAAATT CTGAATTTTG ACAAGGTCTC TGCTGTTGAA ATGGTAAATT 2640
TATTATTTTT TTTGTCATGA TAAATTCTGG TTCAAGGTAT GCTATCCATG AAATAATTTC 2700
TGACCAAAAC TAAATTGATG CAATTTGATT ATCCATCTTA GCCTACAGAT GGCATCTGGT 2760
AACTTTTGAC TGTTTTAAAA AATAAATCCA CTATCAGAGT AGATTTGATG TTGGCTTCAG 2820
AAACATTTAG AAAAACAAAA GTTCAAAAAT GTTTTCAGGA GGTGATAAGT TGAATAACTC 2880
TACAATGTTA GTTCTTTGAG GGGGACAAAA AATTTAAAAT CTTTGAAAGG TCTTATTTTA 2940
CAGCCATATC TAAATTATCT TAAGAAAATT TTTAACAAAG GGAATGAAAT ATATATCATG 3000
ATTCTGTTTT TCCAAAAGTA ACCTGAATAT AGCAATGAAG TTCAGTTTTG TTATTGGTAG 3060
TTTGGGCAGA GTCTCTTTTT GCAGCACCTG TTGTCTACCA TAATTACAGA GGACATTTCC 3120
ATGTTCTAGC CAAGTATACT ATTAGAATAA AAAAACTTAA CATTGAGTTG CTTCAACAGC 3180
ATGAAACTGA GTCCAAAAGA CCAAATGAAC AAACACATTA ATCTCTGATT ATTTATTTTA 3240
AATAGAATAT TTAATTGTGT AAGATCTAAT AGTATCATTA TACTTAAGCA ATCATATTCC 3300
TGATGATCTA TGGGAAATAA CTATTATTTA ATTAATATTG AAACCAGGTT TTAAGATGTG 3360
TTAGCCAGTC CTGTTACTAG TAAATCTCTT TATTTGGAGA GAAATTTTAG ATTGTTTTGT 3420
TCTCCTTATT AGAAGGATTG TAGAAAGAAA AAAATGACTA ATTGGAGAAA AATTGGGGAT 3480
ATATCATATT TCACTGAATT CAAAATGTCT TCAGTTGTAA ATCTTACCAT TATTTTACGT 3540
ACCTCTAAGA AATAAAAGTG CTTCTAATTA AAATATGATG TCATTAATTA TGAAATACTT 3600
CTTGATAACA GAAGTTTTAA AATAGCCATC TTAGAATCAG TGAAATATGG TAATGTATTA 3660
TTTTCCTCCT TTGAGTNAGG TCTTGTGCTT TTTNTTCCTG GCCACTAAAT NTCACCATNT 3720
CCAANAAGCA AANTAAACCT ATTCTGAATA TTTTTGCTGT GAAACACTTG NCAGCAGAGC 3780
TTTCCCNCCA TGNNAGAAGC TTCATGAGTC ACACATTACA TCTTTGGGTT GATTGAATGC 3840
CACTGAAACA TTTCTAGTAG CCTGGAGNAG TTGACCTACC TGTGGAGATG CCTGCCATTA 3900
AATGGCATCC TGATGGCTTA ATACACATCA CTCTTCTGTG NAGGGTTTTA ATTTTCAACA 3960
CAGCTTACTC TGTAGCATCA TGTTTACATT GTATGTATAA AGATTATACN AAGGTGCAAT 4020
TGTGTATTTC TTCCTTAAAA TGTATCAGTA TAGGATTTAG AATCTCCATG TTGAAACTCT 4080
AAATGCATAG AAATAAAAAT AATAAAAAAT TTTTCATTTT GGCTTTTCAG CCTAGTATTA 4140
AAACTGATAA AAGCAAAGCC ATGCACAAAA CTACCTCCCT AGAGAAAGGC TAGTCCCTTT 4200
TCTTCCCCAT TCATTTCATT ATGAACATAG TAGAAAACAG CATATTCTTA TCAAATTTGA 4260
TGAAAAGCGC CAACACGTTT GAACTGAAAT ACGACTTGTC ATGTGAACTG TACCGAATGT 4320
CTACGTATTC CACTTTTCCT GCTGGGGTTC CTGTCTCAGA AAGGAGTCTT GCTCGTGCTG 4380
GTTTCTATTA CACTGGTGTG AATGACAAGG TCAAATGCTT CTGTTGTGGC CTGATGCTGG 4440
ATAACTGGAA AAGAGGAGAC AGTCCTACTG AAAAGCATAA AAAGTTGTAT CCTAGCTGCA 4500
GATTCGTTCA GAGTCTAAAT TCCGTTAACA ACTTGGAAGC TACCTCTCAG CCTACTTTTC 4560
CTTCTTCAGT AACACATTCC ACACACTCAT TACTTCCGGG TACAGAAAAC AGTGGATATT 4620

TCCGTGGCTC TTATTCAAAC TCTCCATCAA ATCCTGTAAA CTCCAGAGCA AATCAAGAAT 4680
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TTACTTTTCA GACATGGCCA TTGACTTTTC TGTCGCCAAC AGATCTGGCA CGAGCAGGCT 4800
TTTACTACAT AGGACCTGGA GACAGAGTGG CTTGCTTTGC CTGTGGTGGA AAATTGAGCA 4860
ATTGGGAACC GAAGGATAAT GCTATGTCAG AACACCTGAG ACATTTTCCC AAATGCCCAT 4920
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CACATGCAGC CCGCTTTAAA ACATTCTTTA ACTGGCCCTC TAGTGTTCTA GTTAATCCTG 5040
AGCAGCTTGC AAGTGCGGGT TTTTATTATG TGGGTAACAG TGATGATGTC AAATGCTTTT 5100
GCTGTGATGG TGGACTCAGG TGTTGGGAAT CTGGAGATGA TCCATGGGTT CAACATGCCA 5160
AGTGGTTTCC AAGGTGTGAG TACTTGATAA GAATTAAAGG ACAGGAGTTC ATCCGTCAAG 5220
TTCAAGCCAG TTACCCTCAT CTACTTGAAC AGCTGCTATC CACATCAGAC AGCCCAGGAG 5280
ATGAAAATGC AGAGTCATCA ATTATCCATT TTGAACCTGG AGAAGACCAT TCAGAAGATG 5340
CAATCATGAT GAATACTCCT GTGATTAATG CTGCCGTGGA AATGGGCTTT AGTAGAAGCC 5400
TGGTAAAACA GACAGTTCAG AGAAAAATCC TAGCAACTGG AGAGAATTAT AGACTAGTCA 5460
ATGATCTTGT GTTAGACTTA CTCAATGCAG AAGATGAAAT AAGGGAAGAG GAGAGAGAAA 5520
GAGCAACTGA GGAAAAAGAA TCAAATGATT TATTATTAAT CCGGAAGAAT AGAATGGCAC 5580
TTTTTCAACA TTTGACTTGT GTAATTCCAA TCCTGGATAG TCTACTAACT GCCGGAATTA 5640
TTAATGAACA AGAACATGAT GTTATTAAAC AGAAGACACA GACGTCTTTA CAAGCAAGAG 5700
AACTGATTGA TACGATTTTA GTAAAAGGAA ATATTGCAGC CACTGTATTC AGAAACTCTC 5760
TGCAAGAAGC TGAAGCTGTG TTATATGAGC ATTTATTTGT GCAACAGGAC ATAAAATATA 5820
TTCCCACAGA AGATGTTTCA GATCTACCAG TGGAAGAACA ATTGCGGAGA CTACAAGAAG 5880
AAAGAACATG TAAAGTGTGT ATGGACAAAG AAGTGTCCAT AGTGTTTATT CCTTGTGGTC 5940
ATCTAGTAGT ATGCAAAGAT TGTGCTCCTT CTTTAAGAAA GTGTCCTATT TGTAGGAGTA 6000
CAATCAAGGG TACAGTTCGT ACATTTCTTT CATGAAGAAG AACCAAAACA TCGTCTAAAC 6060
TTTAGAATTA ATTTATTAAA TGTATTATAA CTTTAACTTT TATCCTAATT TGGTTTCCTT 6120
AAAATTTTTA TTTATTTACA ACTCAAAAAA CATTGTTTTG TGTAACATAT TTATATATGT 6180
ATCTAAACCA TATGAACATA TATTTTTTAG AAACTAAGAG AATGATAGGC TTTTGTTCTT 6240
ATGAACGAAA AAGAGGTAGC ACTACAAACA CAATATTCAA TCAAAATTTC AGCATTATTG 6300
AAATTGTAAG TGAAGTAAAA CTTAAGATAT TTGAGTTAAC CTTTAAGAAT TTTAAATATT 6360
TTGGCATTGT ACTAATACCG GGAACATGAA GCCAGGTGTG GTGGTATGTG CCTGTAGTCC 6420
CAGGCTGAGG CAAGAGAATT ACTTGAGCCC AGGAGTTTGA ATCCATCCTG GGCAGCATAC 6480
TGAGACCCTG CCTTTAAAAA CAAACAGAAC AAAAACAAAA CACCAGGGAC ACATTTCTCT 6540
GTCTTTTTTG ATCAGTGTCC TATACATCGA AGGTGTGCAT ATATGTTGAA TCACATTTTA 6600
GGGACATGGT GTTTTTATAA AGAATTCTGT GAGAAAAAAT TTAATAAAGC AACCAAAAAA 6660
AAAAAAAAA 6669

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 604 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Asn Ile Val Glu Asn Ser Ile Phe Leu Ser Asn Leu Met Lys Ser
 1               5                  10                  15
Ala Asn Thr Phe Glu Leu Lys Tyr Asp Leu Ser Cys Glu Leu Tyr Arg
            20                  25                  30
Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro Val Ser Glu Arg
        35                  40                  45
Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys Val
    50                  55                  60
Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Arg Gly Asp
65                  70                  75                  80
Ser Pro Thr Glu Lys His Lys Lys Leu Tyr Pro Ser Cys Arg Phe Val
                85                  90                  95
Gln Ser Leu Asn Ser Val Asn Asn Leu Glu Ala Thr Ser Gln Pro Thr
            100                 105                 110
Phe Pro Ser Ser Val Thr His Ser Thr His Ser Leu Leu Pro Gly Thr
        115                 120                 125
Glu Asn Ser Gly Tyr Phe Arg Gly Ser Tyr Ser Asn Ser Pro Ser Asn
    130                 135                 140
Pro Val Asn Ser Arg Ala Asn Gln Glu Phe Ser Ala Leu Met Arg Ser
145                 150                 155                 160
Ser Tyr Pro Cys Pro Met Asn Asn Glu Asn Ala Arg Leu Leu Thr Phe
                165                 170                 175
Gln Thr Trp Pro Leu Thr Phe Leu Ser Pro Thr Asp Leu Ala Arg Ala
            180                 185                 190
Gly Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys
        195                 200                 205
Gly Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Asn Ala Met Ser Glu
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    210                 215                 220
His Leu Arg His Phe Pro Lys Cys Pro Phe Ile Glu Asn Gln Leu Gln
225                 230                 235                 240
Asp Thr Ser Arg Tyr Thr Val Ser Asn Leu Ser Met Gln Thr His Ala
                245                 250                 255
Ala Arg Phe Lys Thr Phe Phe Asn Trp Pro Ser Ser Val Leu Val Asn
            260                 265                 270
Pro Glu Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Asn Ser Asp
        275                 280                 285
Asp Val Lys Cys Phe Cys Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser
    290                 295                 300
Gly Asp Asp Pro Trp Val Gln His Ala Lys Trp Phe Pro Arg Cys Glu
305                 310                 315                 320
Tyr Leu Ile Arg Ile Lys Gly Gln Glu Phe Ile Arg Gln Val Gln Ala
                325                 330                 335
Ser Tyr Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Ser Pro
            340                 345                 350
Gly Asp Glu Asn Ala Glu Ser Ser Ile Ile His Leu Glu Pro Gly Glu
        355                 360                 365
Asp His Ser Glu Asp Ala Ile Met Met Asn Thr Pro Val Ile Asn Ala
    370                 375                 380
Ala Val Glu Met Gly Phe Ser Arg Ser Leu Val Lys Gln Thr Val Gln
385                 390                 395                 400
Arg Lys Ile Leu Ala Thr Gly Glu Asn Tyr Arg Leu Val Asn Asp Leu
                405                 410                 415
Val Leu Asp Leu Leu Asn Ala Glu Asp Glu Ile Arg Glu Glu Glu Arg
            420                 425                 430
Glu Arg Ala Thr Glu Glu Lys Glu Ser Asn Asp Leu Leu Leu Ile Arg
        435                 440                 445
Lys Asn Arg Met Ala Leu Phe Gln His Leu Thr Cys Val Ile Pro Ile
    450                 455                 460
Leu Asp Ser Leu Leu Thr Ala Gly Ile Ile Asn Glu Gln Glu His Asp
465                 470                 475                 480
Val Ile Lys Gln Lys Thr Gln Thr Ser Leu Gln Ala Arg Glu Leu Ile
                485                 490                 495
Asp Thr Ile Leu Val Lys Gly Asn Ile Ala Ala Thr Val Phe Arg Asn
            500                 505                 510
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Ser Leu Gln Glu Ala Glu Ala Val Leu Tyr Glu His Leu Phe Val Gln
515 520 525
Gln Asp Ile Lys Tyr Ile Pro Thr Glu Asp Val Ser Asp Leu Pro Val
530 535 540
Glu Glu Gln Leu Arg Arg Leu Pro Glu Glu Arg Thr Cys Lys Val Cys
545 550 555 560
Met Asp Lys Glu Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val
565 570 575
Val Cys Lys Asp Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg
580 585 590
Ser Thr Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
595 600

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3732 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGCGCCCGG GCTGATCCGA GCCGAGCGGG CCGTATCTCC TTGTCGGCGC CGCTGATTCC 60
CGGCTCTGCG GAGGCCTCTA GGCAGCCGCG CAGCTTCCGT GTTTGCTGCG CCCGCACTGC 120
GATTTACAAC CCTGAAGAAT CTCCCTATCC CTATTTTGTC CCCCTGCAGT AATAAATCCC 180
ATTATGGAGA TCTCGAAACT TTATAAAGGG ATATAGTTTG AATTCTATGG AGTGTAATTT 240
TGTGTATGAA TTATATTTTT AAAACATTGA AGAGTTTTCA GAAAGAAGGC TAGTAGAGTT 300
GATTACTGAT ACTTTATGCT AAGCAGTACT TTTTTGGTAG TACAATATTT TGTTAGGCGT 360
TTCTGATAAC ACTAGAAAGG ACAAGTTTTA TCTTGTGATA AATTGATTAA TGTTTACAAC 420
ATGACTGATA ATTATAGCTG AATAGTCCTT AAATGATGAA CAGGTTATTT AGTTTTTAAA 480
TGCAGTGTAA AAAGTGTGCT GTGGAAATTT TATGGCTAAC TAAGTTTATG GAGAAAATAC 540
CTTCAGTTGA TCAAGAATAA TAGTGGTATA CAAAGTTAGG AAGAAAGTCA ACATGATGCT 600
GCAGGAAATG GAAACAAATA CAAATGATAT TTAACAAAGA TAGAGTTTAC AGTTTTTGAA 660
CTTTAAGCCA AATTCATTTG ACATCAAGCA CTATAGCAGG CACAGGTTCA ACAAAGCTTG 720

TGGGTATTGA CTTCCCCCAA AAGTTGTCAG CTGAAGTAAT TTAGCCCACT TAAGTAAATA 780
CTATGATGAT AAGCTGTGTG AACTTAGCTT TTAAATAGTG TGACCATATG AAGGTTTTAA 840
TTACTTTTGT TTATTGGAAT AAAATGAGAT TTTTTGGGTT GTCATGTTAA AGTGCTTATA 900
GGGAAAGAAG CCTGCATATA ATTTTTTACC TTGTGGCATA ATCAGTAATT GGTCTGTTAT 960
TCAGGCTTCA TAGCTTGTAA CCAAATATAA ATAAAAGGCA TAATTTAGGT ATTCTATAGT 1020
TGCTTAGAAT TTTGTTAATA TAAATCTCTG TGAAAAATCA AGGAGTTTTA ATATTTTCAG 1080
AAGTGCATCC ACCTTTCAGG GCTTTAAGTT AGTATTAACT CAAGATTATG AACAAATAGC 1140
ACTTAGGTTA CCTGAAAGAG TTACTACAAC CCCAAAGAGT TGTGTTCTAA GTAGTATCTT 1200
GGTAATTCAG AGAGATACTC ATCCTACCTG AATATAAACT GAGATAAATC CAGTAAAGAA 1260
AGTGTAGTAA ATTCTACATA AGAGTCTATC ATTGATTTCT TTTTGTGGTA AAAATCTTAG 1320
TTCATGTGAA GAAATTTCAT GTGAATGTTT TAGCTATCAA ACAGTACTGT CACCTACTCA 1380
TGCACAAAAC TGCCTCCCAA AGACTTTTCC CAGGTCCCTC GTATCAAAAC ATTAAGAGTA 1440
TAATGGAAGA TAGCACGATC TTGTCAGATT GGACAAACAG CAACAAACAA AAAATGAAGT 1500
ATGACTTTTC CTGTGAACTC TACAGAATGT CTACATATTC AACTTTCCCC GCCGGGGTGC 1560
CTGTCTCAGA AAGGAGTCTT GCTCGTGCTG GTTTTTATTA TACTGGTGTG AATGACAAGG 1620
TCAAATGCTT CTGTTGTGGC CTGATGCTGG ATAACTGGAA ACTAGGAGAC AGTCCTATTC 1680
AAAAGCATAA ACAGCTATAT CCTAGCTGTA GCTTTATTCA GAATCTGGTT TCAGCTAGTC 1740
TGGGATCCAC CTCTAAGAAT ACGTCTCCAA TGAGAAACAG TTTTGCACAT TCATTATCTC 1800
CCACCTTGGA ACATAGTAGC TTGTTCAGTG GTTCTTACTC CAGCCTTTCT CCAAACCCTC 1860
TTAATTCTAG AGCAGTTGAA GACATCTCTT CATCGAGGAC TAACCCCTAC AGTTATGCAA 1920
TGAGTACTGA AGAAGCCAGA TTTCTTACCT ACCATATGTG GCCATTAACT TTTTTGTCAC 1980
CATCAGAATT GGCAAGAGCT GGTTTTTATT ATATAGGACC TGGAGATAGG GTAGCCTGCT 2040
TTGCCTGTGG TGGGAAGCTC AGTAACTGGG AACCAAAGGA TGATGCTATG TCAGAACACC 2100
GGAGGCATTT TCCCAACTGT CCATTTTTGG AAAATTCTCT AGAAACTCTG AGGTTTAGCA 2160
TTTCAAATCT GAGCATGCAG ACACATGCAG CTCGAATGAG AACATTTATG TACTGGCCAT 2220
CTAGTGTTCC AGTTCAGCCT GAGCAGCTTG CAAGTGCTGG TTTTTATTAT GTGGGTCGCA 2280
ATGATGATGT CAAATGCTTT TGTTGTGATG GTGGCTTGAG GTGTTGGGAA TCTGGAGATG 2340
ATCCATGGGT AGAACATGCC AAGTGGTTTC CAAGGTGTGA GTTCTTGATA CGAATGAAAG 2400
GCCAAGAGTT TGTTGATGAG ATTCAAGGTA GATATCCTCA TCTTCTTGAA CAGCTGTTGT 2460
CAACTTCAGA TACCACTGGA GAAGAAAATG CTGACCCACC AATTATTCAT TTTGGACCTG 2520
GAGAAAGTTC TTCAGAAGAT GCTGTCATGA TGAATACACC TGTGGTTAAA TCTGCCTTGG 2580
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GAGAGAACTA TAAAACAGTT AATGATATTG TGTCAGCACT TCTTAATGCT GAAGATGAAA 2700
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CCAACATCTT CAAAAACTGT CTAAAAGAAA TTGACTCTAC ATTGTATAAG AACTTATTTG 3000
TGGATAAGAA TATGAAGTAT ATCCCAACAG AAGATGTTTC AGGTCTGTCA CTGGAAGAAC 3060
AATTGAGGAG GTTGCAAGAA GAACGAACTT GTAAAGTGTG TATGGACAAA GAAGTTTCTG 3120
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AATGCCCTAT TTGCAGGGGT ATAATCAAGG GTACTGTTCG TACATTTCTC TCTTAAAGAA 3240
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CCATCTAAAG TAAAAAGGGA ATTATGAGTT TTTCAATTAG TAACATTCAT GTTCTAGTCT 3360
GCTTTGGTAC TAATAATCTT GTTTCTGAAA AGATGGTATC ATATATTTAA TCTTAATCTG 3420
TTTATTTACA AGGGAAGATT TATGTTTGGT GAACTATATT AGTATGTATG TGTACCTAAG 3480
GGAGTAGTGT CACTGCTTGT TATGCATCAT TTCAGGAGTT ACTGGATTTG TTGTTCTTTC 3540
AGAAAGCTTT GAATACTAAA TTATAGTGTA GAAAAGAACT GGAAACCAGG AACTCTGGAG 3600
TTCATCAGAG TTATGGTGCC GAATTGTCTT TGGTGCTTTT CACTTGTGTT TTAAAATAAG 3660
GATTTTTCTC TTATTTCTCC CCCTAGTTTG TGAGAAACAT CTCAATAAAG TGCTTTAAAA 3720
AGAAAAAAAA AA 3732

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 618 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met His Lys Thr Ala Ser Gln Arg Leu Phe Pro Gly Pro Ser Tyr Gln
1 5 10 15
Asn Ile Lys Ser Ile Met Glu Asp Ser Thr Ile Leu Ser Asp Trp Thr
20 25 30
Asn Ser Asn Lys Gln Lys Met Lys Tyr Asp Phe Ser Cys Glu Leu Tyr
35 40 45
Arg Met Ser Thr Tyr Ser Thr Phe Pro Ala Gly Val Pro Val Ser Glu
50 55 60
Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys
65 70 75 80

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Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Leu Gly
                85                  90                  95
Asp Ser Pro Ile Gln Lys His Lys Gln Leu Tyr Pro Ser Cys Ser Phe
            100                 105                 110
Ile Gln Asn Leu Val Ser Ala Ser Leu Gly Ser Thr Ser Lys Asn Thr
        115                 120                 125
Ser Pro Met Arg Asn Ser Phe Ala His Ser Leu Ser Pro Thr Leu Glu
    130                 135                 140
His Ser Ser Leu Phe Ser Gly Ser Tyr Ser Ser Leu Pro Pro Asn Pro
145                 150                 155                 160
Leu Asn Ser Arg Ala Val Glu Asp Ile Ser Ser Ser Arg Thr Asn Pro
                165                 170                 175
Tyr Ser Tyr Ala Met Ser Thr Glu Glu Ala Arg Phe Leu Thr Tyr His
            180                 185                 190
Met Trp Pro Leu Thr Phe Leu Ser Pro Ser Glu Leu Ala Arg Ala Gly
        195                 200                 205
Phe Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys Gly
    210                 215                 220
Gly Lys Leu Ser Asn Trp Glu Pro Lys Asp Asp Ala Met Ser Glu His
225                 230                 235                 240
Arg Arg His Phe Pro Asn Cys Pro Phe Leu Glu Asn Ser Leu Glu Thr
                245                 250                 255
Leu Arg Phe Ser Ile Ser Asn Leu Ser Met Gln Thr His Ala Ala Arg
            260                 265                 270
Met Arg Thr Phe Met Tyr Trp Pro Ser Ser Val Pro Val Gln Pro Glu
        275                 280                 285
Gln Leu Ala Ser Ala Gly Phe Tyr Tyr Val Gly Arg Asn Asp Asp Val
    290                 295                 300
Lys Cys Phe Gly Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser Gly Asp
305                 310                 315                 320
Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg Cys Glu Phe Leu
                325                 330                 335
Ile Arg Met Lys Gly Gln Glu Phe Val Asp Glu Ile Gln Gly Arg Tyr
            340                 345                 350
Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Thr Thr Gly Glu
        355                 360                 365
Glu Asn Ala Asp Pro Pro Ile Ile His Phe Gly Pro Gly Glu Ser Ser
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370 375 380

Ser Glu Asp Ala Val Met Met Asn Thr Pro Val Val Lys Ser Ala Leu
385 390 395 400
Glu Met Gly Phe Asn Arg Asp Leu Val Lys Gln Thr Val Leu Ser Lys
405 410 415
Ile Leu Thr Thr Gly Glu Asn Tyr Lys Thr Val Asn Asp Ile Val Ser
420 425 430
Ala Leu Leu Asn Ala Glu Asp Glu Lys Arg Glu Glu Glu Lys Glu Lys
435 440 445
Gln Ala Glu Glu Met Ala Ser Asp Asp Leu Ser Leu Ile Arg Lys Asn
450 455 460
Arg Met Ala Leu Phe Gln Gln Leu Thr Cys Val Leu Pro Ile Leu Asp
465 470 475 480
Asn Leu Leu Lys Ala Asn Val Ile Asn Lys Gln Glu His Asp Ile Ile
485 490 495
Lys Gln Lys Thr Gln Ile Pro Leu Gln Ala Arg Glu Leu Ile Asp Thr
500 505 510
Ile Trp Val Lys Gly Asn Ala Ala Ala Asn Ile Phe Lys Asn Cys Leu
515 520 525
Lys Glu Ile Asp Ser Thr Leu Tyr Lys Asn Leu Phe Val Asp Lys Asn
530 535 540
Met Lys Tyr Ile Pro Thr Glu Asp Val Ser Gly Leu Ser Leu Glu Glu
545 550 555 560
Gln Leu Arg Arg Leu Gln Glu Glu Arg Thr Cys Lys Val Cys Met Asp
565 570 575
Lys Glu Val Ser Val Val Phe Ile Pro Cys Gly His Leu Val Val Cys
580 585 590
Gln Glu Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg Gly Ile
595 600 605
Ile Lys Gly Thr Val Arg Thr Phe Leu Ser
610 615

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2691 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATTTTTTAAA TTGATGCATT AACATTCTAA ACATTCATCT GTTTTTAAAT AGTAAAAATT 60
GAACTTTGCC TTGAATATGT AATGATTCAT TATAACAATT ATGCATAGTC TTTAATAATC 120
TGCATATTTT ATGCTGCTTT CATGTTTTTC CTAATTAATG ACTTCACATG TTTAATATTT 180
ATAATTTTTC TGTCATAGTT TCCATATTTA TATAAAATGA ATACTTAAGA TCAGTAATTC 240
TGCTCTGTTT GTTTATATAC TATTTTCCAT CAAAAGACAA AATGGGACTG AGGTTGAGGC 300
TCGTTGCTAA AGCACTTTCC TAAAATGCAA AAGGCCCTAT GATGGATCCC TAGTACTTAT 360
TTAAGTGAGA GAGAAACAGG CTGGGGGTGT AGGTCTGTTA GAGCATGTGT TTGGCATTAT 420
GTGAAGCCCA AACACTAAAA AAGGAGAACA AACAAAAGCG CAGACTTTAA AACTCAAGTG 480
GTTTGGTAAT GTACGACTCT ACTGTTTAGA ATTAAAATGT GTCTTAGTTA TTGTGCCATT 540
ATTTTTATGT CATCACTGGA TAATATATTA GTGCTTAGTA TCAGAAATAG TCCTTATGCT 600
TTGTGTTTTG AAGTTCCTAA TGCAATGTTC TCTTTCTAGA AAAGGTGGAC AAGTCCTATT 660
TTCCAGAGAA GATGACTTTT AACAGTTTTG AAGGAACTAG AACTTTTGTA CTTGCAGACA 720
CCAATAAGGA TGAAGAATTT GTAGAAGAGT TTAATAGATT AAAAACATTT GCTAACTTCC 780
CAAGTAGTAG TCCTGTTTCA GCATCAACAT TGGCGCGAGC TGGGTTTCTT TATACCGGTG 840
AAGGAGACAC CGTGCAATGT TTCAGTTGTC ATGCGGCAAT AGATAGATGG CAGTATGGAG 900
ACTCAGCTGT TGGAAGACAC AGGAGAATAT CCCCAAATTG CAGATTTATC AATGGTTTTT 960
ATTTTGAAAA TGGTGCTGCA CAGTCTACAA ATCCTGGTAT CCAAAATGGC CAGTACAAAT 1020
CTGAAAACTG TGTGGGAAAT AGAAATCCTT TTGCCCCTGA CAGGCCACCT GAGACTCATG 1080
CTGATTATCT CTTGAGAACT GGACAGGTTG TAGATATTTC AGACACCATA TACCCGAGGA 1140
ACCCTGCCAT GTGTAGTGAA GAAGCCAGAT TGAAGTCATT TCAGAACTGG CCGGACTATG 1200
CTCATTTAAC CCCCAGAGAG TTAGCTAGTG CTGGCCTCTA CTACACAGGG GCTGATGATC 1260
AAGTGCAATG CTTTTGTTGT GGGGGAAAAC TGAAAAATTG GGAACCCTGT GATCGTGCCT 1320
GGTCAGAACA CAGGAGACAC TTTCCCAATT GCTTTTTTGT TTTGGGCCGG AACGTTAATG 1380
TTCGAAGTGA ATCTGGTGTG AGTTCTGATA GGAATTTCCC AAATTCAACA AACTCTCCAA 1440
GAAATCCAGC CATGGCAGAA TATGAAGCAC GGATCGTTAC TTTTGGAACA TGGACATCCT 1500
CAGTTAACAA GGAGCAGCTT GCAAGAGCTG GATTTTATGC TTTAGGTGAA GGCGATAAAG 1560
TGAAGTGCTT CCACTGTGGA GGAGGGCTCA CGGATTGGAA GCCAAGTGAA GACCCCTGGG 1620
ACCAGCATGC TAAGTGCTAC CCAGGGTGCA AATACCTATT GGATGAGAAG GGGCAAGAAT 1680
ATATAAATAA TATTCATTTA ACCCATCCAC TTGAGGAATC TTTGGGAAGA ACTGCTGAAA 1740
AAACACCACC GCTAACTAAA AAAATCGATG ATACCATCTT CCAGAATCCT ATGGTGCAAG 1800

AAGCTATACG AATGGGATTT AGCTTCAAGG ACCTTAAGAA AACAATGGAA GAAAAAATCC 1860
AAACATCCGG GAGCAGCTAT CTATCACTTG AGGTCCTGAT TGCAGATCTT GTGAGTGCTC 1920
AGAAAGATAA TACGGAGGAT GAGTCAAGTC AAACTTCATT GCAGAAAGAC ATTAGTACTG 1980
AAGAGCAGCT AAGGCGCCTA CAAGAGGAGA AGCTTTCCAA AATCTGTATG GATAGAAATA 2040
TTGCTATCGT TTTTTTTCCT TGTGGACATC TGGCCACTTG TAAACAGTGT GCAGAAGCAG 2100
TTGACAAATG TCCCATGTGC TACACCGTCA TTACGTTCAA CCAAAAAATT TTTATGTCTT 2160
AGTGGGGCAC CACATGTTAT GTTCTTCTTG CTCTAATTGA ATGTGTAATG GGAGCGAACT 2220
TTAAGTAATC CTGCATTTGC ATTCCATTAG CATCCTGCTG TTTCCAAATG GAGACCAATG 2280
CTAACAGCAC TGTTTCCGTC TAAACATTCA ATTTCTGGAT CTTTCGAGTT ATCAGCTGTA 2340
TCATTTAGCC AGTGTTTTAC TCGATTGAAA CCTTAGACAG AGAAGCATTT TATAGCTTTT 2400
CACATGTATA TTGGTAGTAC ACTGACTTGA TTTCTATATG TAAGTGAATT CATCACCTGC 2460
ATGTTTCATG CCTTTTGCAT AAGCTTAACA AATGGAGTGT TCTGTATAAG CATGGAGATG 2520
TGATGGAATC TGCCCAATGA CTTTAATTGG CTTATTGTAA ACACGGAAAG AACTGCCCCA 2580
CGCTGCTGGG AGGATAAAGA TTGTTTTAGA TGCTCACTTC TGTGTTTTAG GATTCTGCCC 2640
ATTTACTTGG AATTTATTGG AGTTATAATG TACTTATATG ATATTTCCGA A 2691

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 496 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Thr Phe Asn Ser Phe Glu Gly Thr Arg Thr Phe Val Leu Ala Asp
1 5 10 15
Thr Asn Lys Asp Glu Glu Phe Val Glu Glu Phe Asn Arg Leu Lys Thr
20 25 30
Phe Ala Asn Phe Pro Ser Ser Ser Pro Val Ser Ala Ser Thr Leu Ala
35 40 45
Arg Ala Gly Phe Leu Tyr Thr Gly Glu Gly Asp Thr Val Gln Cys Phe
50 55 60
Ser Cys His Ala Ala Ile Asp Arg Trp Gln Tyr Gly Asp Ser Ala Val

65 70 75 80
Gly Arg His Arg Arg Ile Ser Pro Asn Cys Arg Phe Ile Asn Gly Phe
85 90 95
Tyr Phe Glu Asn Gly Ala Ala Gln Ser Thr Asn Pro Gly Ile Gln Asn
100 105 110
Gly Gln Tyr Lys Ser Glu Asn Cys Val Gly Asn Arg Asn Pro Phe Ala
115 120 125
Pro Asp Arg Pro Pro Glu Thr His Ala Asp Tyr Leu Leu Arg Thr Gly
130 135 140
Gln Val Val Asp Ile Ser Asp Thr Ile Tyr Pro Arg Asn Pro Ala Met
145 150 155 160
Cys Ser Glu Glu Ala Arg Leu Lys Ser Phe Gln Asn Trp Pro Asp Tyr
165 170 175
Ala His Leu Thr Pro Arg Glu Leu Ala Ser Ala Gly Leu Tyr Tyr Thr
180 185 190
Gly Ala Asp Asp Gln Val Gln Cys Phe Cys Cys Gly Gly Lys Leu Lys
195 200 205
Asn Trp Glu Pro Cys Asp Arg Ala Trp Ser Glu His Arg Arg His Phe
210 215 220
Pro Asn Cys Phe Phe Val Leu Gly Arg Asn Val Asn Val Arg Ser Glu
225 230 235 240
Ser Gly Val Ser Ser Asp Arg Asn Phe Pro Asn Ser Thr Asn Ser Pro
245 250 255
Arg Asn Pro Ala Met Ala Glu Tyr Glu Ala Arg Ile Val Thr Phe Gly
260 265 270
Thr Trp Ile Tyr Ser Val Asn Lys Glu Gln Leu Ala Arg Ala Gly Phe
275 280 285
Tyr Ala Leu Gly Glu Gly Asp Lys Val Lys Cys Phe His Cys Gly Gly
290 295 300
Gly Leu Thr Asp Trp Lys Pro Ser Glu Asp Pro Trp Asp Gln His Ala
305 310 315 320
Lys Cys Tyr Pro Gly Cys Lys Tyr Leu Leu Asp Glu Lys Gly Gln Glu
325 330 335
Tyr Ile Asn Asn Ile His Leu Thr His Pro Leu Glu Glu Ser Leu Gly
340 345 350
Arg Thr Ala Glu Lys Thr Pro Pro Leu Thr Lys Lys Ile Asp Asp Thr
355 360 365

Ile Phe Gln Asn Pro Met Val Gln Glu Ala Ile Arg Met Gly Phe Ser
370 375 380
Phe Lys Asp Leu Lys Lys Thr Met Glu Glu Lys Ile Gln Thr Ser Gly
385 390 395 400
Ser Ser Tyr Leu Ser Leu Glu Val Leu Ile Ala Asp Leu Val Ser Ala
405 410 415
Gln Lys Asp Asn Thr Glu Asp Glu Ser Ser Gln Thr Ser Leu Gln Lys
420 425 430
Asp Ile Ser Thr Glu Glu Gln Leu Arg Arg Leu Gln Glu Glu Lys Leu
435 440 445
Ser Lys Ile Cys Met Asp Arg Asn Ile Ala Ile Val Phe Phe Pro Cys
450 455 460
Gly His Leu Ala Thr Cys Lys Gln Cys Ala Glu Ala Val Asp Lys Cys
465 470 475 480
Pro Met Cys Tyr Thr Val Ile Thr Phe Asn Gln Lys Ile Phe Met Ser
485 490 495

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2676 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGGGAGTTCC CCGGAGCCCT GGAGGAAAGC ACCGCAGGTC TGAGCAGCCC TGAGCCGGGC 60
AGGGTGGGGG CAGTGGCTAA GGCCTAGCTG GGGACGATTT AAAGGTATCG CGCCACCCAG 120
CCACACCCCA CAGGCCAGGC GAGGGTGCCA CCCCCGGAGA TCAGAGGTCA TTGCTGGCGT 180
TCAGAGCCTA GGAAGTGGGC TGCGGTATCA GCCTAGCAGT AAAACCGACC AGAAGCCATG 240
CACAAAACTA CATCCCCAGA GAAAGACTTG TCCCTTCCCC TCCCTGTCAT CTCACCATGA 300
ACATGGTTCA AGACAGCGCC TTTCTAGCCA AGCTGATGAA GAGTGCTGAC ACCTTTGAGT 360
TGAAGTATGA CTTTTCCTGT GAGCTGTACC GATTGTCCAC GTATTCAGCT TTTCCCAGGG 420
GAGTTCCTGT GTCAGAAAGG AGTCTGGCTC GTGCTGGCTT TTACTACACT GGTGCCAATG 480

ACAAGGTCAA GTGCTTCTGC TGTGGCCTGA TGCTAGACAA CTGGAAACAA GGGGACAGTC 540
CCATGGAGAA GCACAGAAAG TTGTACCCCA GCTGCAACTT TGTACAGACT TTGAATCCAG 600
CCAACAGTCT GGAAGCTAGT CCTCGGCCTT CTCTTCCTTC CACGGCGATG AGCACCATGC 660
CTTTGAGCTT TGCAAGTTCT GAGAATACTG GCTATTTCAG TGGCTCTTAC TCGAGCTTTC 720
CCTCAGACCC TGTGAACTTC CGAGCAAATC AAGATTGTCC TGCTTTGAGC ACAAGTCCCT 780
ACCACTTTGC AATGAACACA GAGAAGGCCA GATTACTCAC CTATGAAACA TGGCCATTGT 840
CTTTTCTGTC ACCAGCAAAG CTGGCCAAAG CAGGCTTCTA CTACATAGGA CCTGGAGATA 900
GAGTGGCCTG CTTTGCGTGC GATGGGAAAC TGAGCAACTG GGAACGTAAG GATGATGCTA 960
TGTCAGAGCA CCAGAGGCAT TTCCCCAGCT GTCCGTTCTT AAAAGACTTG GGTCAGTCTG 1020
CTTCGAGATA CACTGTCTCT AACCTGAGCA TGCAGACACA CGCAGCCCGT ATTAGAACAT 1080
TCTCTAACTG GCCTTCTAGT GCACTAGTTC ATTCCCAGGA ACTTGCAAGT GCGGGCTTTT 1140
ATTATACAGG ACACAGTGAT GATGTCAAGT GTTTTTGCTG TGATGGTGGG CTGAGGTGCT 1200
GGGAATCTGG AGATGACCCC TGGGTGGAAC ATGCCAAGTG GTTTCCAAGG TGTGAGTACT 1260
TGCTCAGAAT CAAAGGCCAA GAATTTGTCA GCCAAGTTCA AGCTGGCTAT CCTCATCTAC 1320
TTGAGCAGCT ATTATCTACG TCAGACTCCC CAGAAGATGA GAATGCAGAC GCAGCAATCG 1380
TGCATTTTGG CCCTGGAGAA AGTTCGGAAG ATGTCGTCAT GATGAGCACG CCTGTGGTTA 1440
AAGCAGCCTT GGAAATGGGC TTCAGTAGGA GCCTGGTGAG ACAGACGGTT CAGCGGCAGA 1500
TCCTGGCCAC TGGTGAGAAC TACAGGACCG TCAGTGACCT CGTTATAGGC TTACTCGATG 1560
CAGAAGACGA GATGAGAGAG GAGCAGATGG AGCAGGCGGC CGAGGAGGAG GAGTCAGATG 1620
ATCTAGCACT AATCCGGAAG AACAAAATGG TGCTTTTCCA ACATTTGACG TGTGTGACAC 1680
CAATGCTGTA TTGCCTCCTA AGTGCAAGGG CCATCACTGA ACAGGAGTGC AATGCTGTGA 1740
AACAGAAACC ACACACCTTA CAAGCAAGCA CACTGATTGA TACTGTGTTA GCAAAAGGAA 1800
ACACTGCAGC AACCTCATTC AGAAACTCCC TTCGGGAAAT TGACCCTGCG TTATACAGAG 1860
ATATATTTGT GCAACAGGAC ATTAGGAGTC TTCCCACAGA TGACATTGCA GCTCTACCAA 1920
TGGAAGAACA GTTGCGGAAA CTCCAGGAGG AAAGAATGTG TAAAGTGTGT ATGGACCGAG 1980
AGGTATCCAT CGTGTTCATT CCCTGTGGCC ATCTGGTCGT GTGCAAAGAC TGCGCTCCCT 2040
CTCTGAGGAA GTGTCCCATC TGTAGAGGGA CCATCAAGGG CACAGTGCGC ACATTTCTCT 2100
CCTGAACAAG ACTAATGGTC CATGGCTGCA ACTTCAGCCA GGAGGAAGTT CACTGTCACT 2160
CCCAGCTCCA TTCGGAACTT GAGGCCAGCC TGGATAGCAC GAGACACCGC CAAACACACA 2220
AATATAAACA TGAAAAACTT TTGTCTGAAG TCAAGAATGA ATGAATTACT TATATAATAA 2280
TTTTAATTGG TTTCCTTAAA AGTGCTATTT GTTCCCAACT CAGAAAATTG TTTTCTGTAA 2340
ACATATTTAC ATACTACCTG CATCTAAAGT ATTCATATAT TCATATATTC AGATGTCATG 2400
AGAGAGGGTT TTGTTCTTGT TCCTGAAAAG CAGGGATTGC CTGCACTCCT GAAATTCTCA 2460
GAAAGATTTA CAATGTTGGC ATTTATGGTT CAGAAACTAG AATCTTCTCC CGTTGCTTTA 2520
AGAACCGGGA GCACAGATGT CCATGTGTTT TATGTATAGA AATTCCTGTT ATTTATTGGA 2580
TGACATTTTA GGGATATGAA ATTTTTATAA AGAATTTGTG AGAAAAAGTT AATAAAGCAA 2640
CATAATTACC TCTTTTTTTT TAAAGAAAAA AAAAAA 2676

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 600 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Val Gln Asp Ser Ala Phe Leu Ala Lys Leu Met Lys Ser Ala Asp
 1               5                  10                  15
Thr Phe Glu Leu Lys Tyr Asp Phe Ser Cys Glu Leu Tyr Arg Leu Ser
            20                  25                  30
Thr Tyr Ser Ala Phe Pro Arg Gly Val Pro Val Ser Glu Arg Ser Leu
        35                  40                  45
Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Ala Asn Asp Lys Val Lys Cys
    50                  55                  60
Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Gln Gly Asp Ser Pro
65                  70                  75                  80
Met Glu Lys His Arg Lys Leu Tyr Pro Ser Cys Asn Phe Val Gln Thr
                85                  90                  95
Leu Asn Pro Ala Asn Ser Leu Glu Ala Ser Pro Arg Pro Ser Leu Pro
            100                 105                 110
Ser Thr Ala Met Ser Thr Met Pro Leu Ser Phe Ala Ser Ser Glu Asn
        115                 120                 125
Thr Gly Tyr Phe Ser Gly Ser Tyr Ser Ser Phe Pro Ser Asp Pro Val
    130                 135                 140
Asn Phe Arg Ala Asn Gln Asp Cys Pro Ala Leu Ser Thr Ser Pro Tyr
145                 150                 155                 160
His Phe Ala Met Asn Thr Glu Lys Ala Arg Leu Leu Thr Tyr Glu Thr
                165                 170                 175
Trp Pro Leu Ser Phe Leu Ser Pro Ala Lys Leu Ala Lys Ala Gly Phe
            180                 185                 190
Tyr Tyr Ile Gly Pro Gly Asp Arg Val Ala Cys Phe Ala Cys Asp Gly
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        195                 200                 205
Lys Leu Ser Asn Trp Glu Arg Lys Asp Asp Ala Met Ser Glu His Gln
    210                 215                 220
Arg His Phe Pro Ser Cys Pro Phe Leu Lys Asp Leu Gly Gln Ser Ala
225                 230                 235                 240
Ser Arg Tyr Thr Val Ser Asn Leu Ser Met Gln Thr His Ala Ala Arg
                245                 250                 255
Ile Arg Thr Phe Ser Asn Trp Pro Ser Ser Ala Leu Val His Ser Gln
            260                 265                 270
Glu Leu Ala Ser Ala Gly Phe Tyr Tyr Thr Gly His Ser Asp Asp Val
        275                 280                 285
Lys Cys Phe Cys Cys Asp Gly Gly Leu Arg Cys Trp Glu Ser Gly Asp
    290                 295                 300
Asp Pro Trp Val Glu His Ala Lys Trp Phe Pro Arg Cys Glu Tyr Leu
305                 310                 315                 320
Leu Arg Ile Lys Gly Gln Glu Phe Val Ser Gln Val Gln Ala Gly Tyr
                325                 330                 335
Pro His Leu Leu Glu Gln Leu Leu Ser Thr Ser Asp Ser Pro Glu Asp
            340                 345                 350
Glu Asn Ala Asp Ala Ala Ile Val His Phe Gly Pro Gly Glu Ser Ser
        355                 360                 365
Glu Asp Val Val Met Met Ser Thr Pro Val Val Lys Ala Ala Leu Glu
    370                 375                 380
Met Gly Phe Ser Arg Ser Leu Val Arg Gln Thr Val Gln Arg Gln Ile
385                 390                 395                 400
Leu Ala Thr Gly Glu Asn Tyr Arg Thr Val Ser Asp Leu Val Ile Gly
                405                 410                 415
Leu Leu Asp Ala Glu Asp Glu Met Arg Glu Glu Gln Met Glu Gln Ala
            420                 425                 430
Ala Glu Glu Glu Glu Ser Asp Asp Leu Ala Leu Ile Arg Lys Asn Lys
        435                 440                 445
Met Val Leu Phe Gln His Leu Thr Cys Val Thr Pro Met Leu Tyr Cys
    450                 455                 460
Leu Leu Ser Ala Arg Ala Ile Thr Glu Gln Glu Cys Asn Ala Val Lys
465                 470                 475                 480
Gln Lys Pro His Thr Leu Gln Ala Ser Thr Leu Ile Asp Thr Val Leu
                485                 490                 495
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Ala Lys Gly Asn Thr Ala Ala Thr Ser Phe Arg Asn Ser Leu Arg Glu
            500                 505                 510
Ile Asp Pro Ala Leu Tyr Arg Asp Ile Phe Val Gln Gln Asp Ile Arg
        515                 520                 525
Ser Leu Pro Thr Asp Asp Ile Ala Ala Leu Pro Met Glu Glu Gln Leu
    530                 535                 540
Arg Lys Leu Gln Glu Glu Arg Met Cys Lys Val Cys Met Asp Arg Glu
545                 550                 555                 560
Val Ser Ile Val Phe Ile Pro Cys Gly His Leu Val Val Cys Lys Asp
                565                 570                 575
Cys Ala Pro Ser Leu Arg Lys Cys Pro Ile Cys Arg Gly Thr Ile Lys
            580                 585                 590
Gly Thr Val Arg Thr Phe Leu Ser
        595                 600
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3151 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

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AGTTATATAA AATACGAAGT TTTCAAAAAG AAGGCTAGTG CAACAGAAAA GCTTTGCTAA     60
AACAGATTCT TAGTTATTTG AGGTAACAAA AGAAAGCCAT GTCTTGAATT GATTCGTTCT    120
TAATTATAAC AGACTTATAG TGGAAAGGGC CTTAAACACA GGCGGACTTT ATAAAATGCA    180
GTCTTAGGTT TATGTGCAAA ATACTGTCTG TTGACCAGAT GTATTCACAT GATATATACA    240
GAGTCAAGGT GGTGATATAG AAGATTTAAC AGTGAGGGAG TTAACAGTCT GTGCTTTAAG    300
CGCAGTTCCT TTACAGTGAA TACTGTAGTC TTAATAGACC TGAGCTGACT GCTGCAGTTG    360
ATGTAACCCA CTTTAGAGAA TACTGTATGA CATCTTCTCT AAGGAAAACC AGCTGCAGAC    420
TTCACTCAGT TCCTTTCATT TCATAGGAAA AGGAGTAGTT CAGATGTCAT GTTTAAGTCC    480
TTATAAGGGA AAAGAGCCTG AATATATGCC CTAGTACCTA GGCTTCATAA CTAGTAATAA    540
GAAGTTAGTT ATGGGTAAAT AGATCTCAGG TTACCCAGAA GAGTTCATGT GACCCCCAAA    600
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GAGTCCTAAC TAGTGTCTTG GCAAGTGAGA CAGATTTGTC CTGTGAGGGT GTCAATTCAC 660

CAGTCCAAGC AGAAGACAAT GAATCTATCC AGTCAGGTGT CTGTGGTGGA GATCTAGTGT 720

CCAAGTGGTG AGAAACTTCA TCTGGAAGTT TAAGCGGTCA GAAATACTAT TACTACTCAT 780

GGACAAAACT GTCTCCCAGA GACTCGGCCA AGGTACCTTA CACCAAAAAC TTAAACGTAT 840

AATGGAGAAG AGCACAATCT TGTCAAATTG GACAAAGGAG AGCGAAGAAA AAATGAAGTT 900

TGACTTTTCG TGTGAACTCT ACCGAATGTC TACATATTCA GCTTTTCCCA GGGGAGTTCC 960

TGTCTCAGAG AGGAGTCTGG CTCGTGCTGG CTTTTATTAT ACAGGTGTGA ATGACAAAGT 1020

CAAGTGCTTC TGCTGTGGCC TGATGTTGGA TAACTGGAAA CAAGGGGACA GTCCTGTTGA 1080

AAAGCACAGA CAGTTCTATC CCAGCTGCAG CTTTGTACAG ACTCTGCTTT CAGCCAGTCT 1140

GCAGTCTCCA TCTAAGAATA TGTCTCCTGT GAAAAGTAGA TTTGCACATT CGTCACCTCT 1200

GGAACGAGGT GGCATTCACT CCAACCTGTG CTCTAGCCCT CTTAATTCTA GAGCAGTGGA 1260

AGACTTCTCA TCAAGGATGG ATCCCTGCAG CTATGCCATG AGTACAGAAG AGGCCAGATT 1320

TCTTACTTAC AGTATGTGGC CTTTAAGTTT TCTGTCACCA GCAGAGCTGG CCAGAGCTGG 1380

CTTCTATTAC ATAGGGCCTG GAGACAGGGT GGCCTGTTTT GCCTGTGGTG GGAAACTGAG 1440

CAACTGGGAA CCAAAGGATG ATGCTATGTC AGAGCACCGC AGACATTTTC CCCACTGTCC 1500

ATTTCTGGAA AATACTTCAG AAACACAGAG GTTTAGTATA TCAAATCTAA GTATGCAGAC 1560

ACACTCTGCT CGATTGAGGA CATTTCTGTA CTGGCCACCT AGTGTTCCTG TTCAGCCCGA 1620

GCAGCTTGCA AGTGCTGGAT TCTATTACGT GGATCGCAAT GATGATGTCA AGTGCTTTTG 1680

TTGTGATGGT GGCTTGAGAT GTTGGGAACC TGGAGATGAC CCCTGGATAG AACACGCCAA 1740

ATGGTTTCCA AGGTGTGAGT TCTTGATACG GATGAAGGGT CAGGAGTTTG TTGATGAGAT 1800

TCAAGCTAGA TATCCTCATC TTCTTGAGCA GCTGTTGTCC ACTTCAGACA CCCCAGGAGA 1860

AGAAAATGCT GACCCTACAG AGACAGTGGT GCATTTTGGC CCTGGAGAAA GTTCGAAAGA 1920

TGTCGTCATG ATGAGCACGC CTGTGGTTAA AGCAGCCTTG GAAATGGGCT TCAGTAGGAG 1980

CCTGGTGAGA CAGACGGTTC AGCGGCAGAT CCTGGCCACT GGTGAGAACT ACAGGACCGT 2040

CAATGATATT GTCTCAGTAC TTTTGAATGC TGAAGATGAG AGAAGAGAAG AGGAGAAGGA 2100

AAGACAGACT GAAGAGATGG CATCAGGTGA CTTATCACTG ATTCGGAAGA ATAGAATGGC 2160

CCTCTTTCAA CAGTTGACAC ATGTCCTTCC TATCCTGGAT AATCTTCTTG AGGCCAGTGT 2220

AATTACAAAA CAGGAACATG ATATTATTAG ACAGAAAACA CAGATACCCT TACAAGCAAG 2280

AGAGCTTATT GACACCGTTT TAGTCAAGGG AAATGCTGCA GCCAACATCT TCAAAAACTC 2340

TCTGAAGGAA ATTGACTCCA CGTTATATGA AAACTTATTT GTGGAAAAGA ATATGAAGTA 2400

TATTCCAACA GAAGACGTTT CAGGCTTGTC ATTGGAAGAG CAGTTGCGGA GATTACAAGA 2460

AGAACGAACT TGCAAAGTGT GTATGGACAG AGAGGTTTCT ATTGTGTTCA TTCCGTGTGG 2520

TCATCTAGTA GTCTGCCAGG AATGTGCCCC TTCTCTAAGG AAGTGCCCCA TCTGCAGGGG 2580

GACAATCAAG GGGACTGTGC GCACATTTCT CTCATGAGTG AAGAATGGTC TGAAAGTATT 2640

GTTGGACATC AGAAGCTGTC AGAACAAAGA ATGAACTACT GATTTCAGCT CTTCAGCAGG 2700

ACATTCTACT CTCTTTCAAG ATTAGTAATC TTGCTTTATG AAGGGTAGCA TTGTATATTT 2760

AAGCTTAGTC TGTTGCAAGG GAAGGTCTAT GCTGTTGAGC TACAGGACTG TGTCTGTTCC 2820

AGAGCAGGAG TTGGGATGCT TGCTGTATGT CCTTCAGGAC TTCTTGGATT TGGAATTTGT 2880
GAAAGCTTTG GATTCAGGTG ATGTGGAGCT CAGAAATCCT GAAACCAGTG GCTCTGGTAC 2940
TCAGTAGTTA GGGTACCCTG TGCTTCTTGG TGCTTTTCCT TTCTGGAAAA TAAGGATTTT 3000
TCTGCTACTG GTAAATATTT TCTGTTTGTG AGAAATATAT TAAAGTGTTT CTTTTAAAGG 3060
CGTGCATCAT TGTAGTGTGT GCAGGGATGT ATGCAGGCAA AACACTGTGT ATATAATAAA 3120
TAAATCTTTT TAAAAAGTGT AAAAAAAAAA A 3151

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 612 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Asp Lys Thr Val Ser Gln Arg Leu Gly Gln Gly Thr Leu His Gln
1 5 10 15
Lys Leu Lys Arg Ile Met Glu Lys Ser Thr Ile Leu Ser Asn Trp Thr
20 25 30
Lys Glu Ser Glu Glu Lys Met Lys Phe Asp Phe Ser Cys Glu Leu Tyr
35 40 45
Arg Met Ser Thr Tyr Ser Ala Phe Pro Arg Gly Val Pro Val Ser Glu
50 55 60
Arg Ser Leu Ala Arg Ala Gly Phe Tyr Tyr Thr Gly Val Asn Asp Lys
65 70 75 80
Val Lys Cys Phe Cys Cys Gly Leu Met Leu Asp Asn Trp Lys Gln Gly
85 90 95
Asp Ser Pro Val Glu Lys His Arg Gln Phe Tyr Pro Ser Cys Ser Phe
100 105 110
Val Gln Thr Leu Leu Ser Ala Ser Leu Gln Ser Pro Ser Lys Asn Met
115 120 125
Ser Pro Val Lys Ser Arg Phe Ala His Ser Ser Pro Leu Glu Arg Gly
130 135 140

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Gly Ile His Ser Asn Leu Cys Ser Ser Pro Leu Asn Ser Arg Ala Val
145                 150                 155                 160
Glu Asp Phe Ser Ser Arg Met Asp Pro Cys Ser Tyr Ala Met Ser Thr
                165                 170                 175
Glu Glu Ala Arg Phe Leu Thr Tyr Ser Met Trp Pro Leu Ser Phe Leu
            180                 185                 190
Ser Pro Ala Glu Leu Ala Arg Ala Gly Phe Tyr Tyr Ile Gly Pro Gly
        195                 200                 205
Asp Arg Val Ala Cys Phe Ala Cys Gly Gly Lys Leu Ser Asn Trp Glu
    210                 215                 220
Pro Lys Asp Asp Ala Met Ser Glu His Arg Arg His Phe Pro His Cys
225                 230                 235                 240
Pro Phe Leu Glu Asn Thr Ser Glu Thr Gln Arg Phe Ser Ile Ser Asn
                245                 250                 255
Leu Ser Met Gln Thr His Ser Ala Arg Leu Arg Thr Phe Leu Tyr Trp
            260                 265                 270
Pro Pro Ser Val Pro Val Gln Pro Glu Gln Leu Ala Ser Ala Gly Phe
        275                 280                 285
Tyr Tyr Val Asp Arg Asn Asp Asp Val Lys Cys Phe Cys Cys Asp Gly
    290                 295                 300
Gly Leu Arg Cys Trp Glu Pro Gly Asp Asp Pro Trp Ile Glu His Ala
305                 310                 315                 320
Lys Trp Phe Pro Arg Cys Glu Phe Leu Ile Arg Met Lys Gly Gln Glu
                325                 330                 335
Phe Val Asp Glu Ile Gln Ala Arg Tyr Pro His Leu Leu Glu Gln Leu
            340                 345                 350
Leu Ser Thr Ser Asp Thr Pro Gly Glu Glu Asn Ala Asp Pro Thr Glu
        355                 360                 365
Thr Val Val His Phe Gly Pro Gly Glu Ser Ser Lys Asp Val Val Met
    370                 375                 380
Met Ser Thr Pro Val Val Lys Ala Ala Leu Glu Met Gly Phe Ser Arg
385                 390                 395                 400
Ser Leu Val Arg Gln Thr Val Gln Arg Gln Ile Leu Ala Thr Gly Glu
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Asn Tyr Arg Thr Val Asn Asp Ile Val Ser Val Leu Leu Asn Ala Glu
            420                 425                 430
Asp Glu Arg Arg Glu Glu Glu Lys Glu Arg Gln Thr Glu Glu Met Ala
```

435 440 445

Ser Gly Asp Leu Ser Leu Ile Arg Lys Asn Arg Met Ala Leu Phe Gln
450 455 460

Gln Leu Thr His Val Leu Pro Ile Leu Asp Asn Leu Leu Glu Ala Ser
465 470 475 480

Val Ile Thr Lys Gln Glu His Asp Ile Ile Arg Gln Lys Thr Gln Ile
485 490 495

Pro Leu Gln Ala Arg Glu Leu Ile Asp Thr Val Leu Val Lys Gly Asn
500 505 510

Ala Ala Ala Asn Ile Phe Lys Asn Ser Leu Lys Glu Ile Asp Ser Thr
515 520 525

Leu Tyr Glu Asn Leu Phe Val Glu Lys Asn Met Lys Tyr Ile Pro Thr
530 535 540

Glu Asp Val Ser Gly Leu Ser Leu Glu Glu Gln Leu Arg Arg Leu Gln
545 550 555 560

Glu Glu Arg Thr Cys Lys Val Cys Met Asp Arg Glu Val Ser Ile Val
565 570 575

Phe Ile Pro Cys Gly His Leu Val Val Cys Gln Glu Cys Ala Pro Ser
580 585 590

Leu Arg Lys Cys Pro Ile Cys Arg Gly Thr Ile Lys Gly Thr Val Arg
595 600 605

Thr Phe Leu Ser
610

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AGTGCGGGTT TTTATTATGT G 21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AGATGACCAC AAGGAATAAA CACTA 25

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu
1 5 10

What is claimed is:

Claims

1. A method for enhancing apoptosis in a cell from a mammal with a proliferative disease, said method comprising administering to said cell a compound that inhibits the biological activity of an IAP polypeptide or an NAIP polypeptide, said compound being administered to said cell in an amount sufficient to enhance apoptosis in said cell.

2. The method of claim 1, wherein said cell is proliferating in said proliferative disease.

3. The method of claim 1, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.

4. The method of claim 3, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

5. The method of claim 1, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

6. The method of claim 1, wherein said polypeptide is NAIP.

7. The method of claim 1, wherein said polypeptide is XIAP.

8. The method of claim 1, wherein said polypeptide is HIAP-1.

9. The method of claim 1, wherein said polypeptide is HIAP-2.

10. The method of claim 1, wherein said compound is a negative regulator of an IAP or an NAIP-dependent anti-apoptotic pathway; wherein said compound is a fragment of said

IAP polypeptide, said fragment comprising a ring zinc finger and having no more than two BIR domains; wherein said compound is a nucleic acid molecule encoding a ring zinc finger domain of said IAP polypeptide; wherein said compound is a compound that prevents cleavage of said IAP polypeptide or said NAIP polypeptide; wherein said compound is a purified antibody or a fragment thereof that specifically binds to said IAP polypeptide or said NAIP polypeptide; wherein said compound is a ribozyme; or wherein said compound is an antisense nucleic acid molecule have a nucleic acid sequence that is complementary to the coding strand of a nucleic acid sequence encoding said IAP polypeptide or said NAIP polypeptide.

11. The method of claim 10, wherein said cleavage is decreased by at least 20% in said cell.

12. The method of claim 10, wherein said antibody binds to a BIR domain of said IAP polypeptide or said NAIP polypeptide.

13. The method of claim 10, wherein said nucleic acid sequence encoding said IAP polypeptide or said NAIP polypeptide has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, or the nucleic acid sequence of NAIP.

14. The method of claim 10, wherein said antisense nucleic acid molecule decreases the level of said nucleic acid sequence encoding said IAP polypeptide or said NAIP polypeptide by at least 20%, said level being measured in the cytoplasm of said cell.

15. The method of claim 10, wherein said antisense nucleic acid molecule is encoded by a virus vector.

16. The method of claim 10, wherein said antisense nucleic acid molecule is encoded by a transgene.

17. The method of claim 1, wherein said mammal is a human or a mouse.

18. The method of claim 1, wherein said proliferative disease is cancer.

19. The method of claim 18, wherein said cancer is in a tissue selected from the group consisting of ovary, breast, pancreas, lymph node, skin, blood, lung, brain, kidney, liver, nasopharyngeal cavity, thyroid, central nervous system, prostate, colon, rectum, cervix, endometrium, and lung.

20. A method for detecting a proliferative disease or an increased likelihood of said proliferative disease in a mammal, said method comprising:

(a) contacting an IAP or a NAIP nucleic acid molecule that is greater than about 18 nucleotides in length with a preparation of nucleic acid from a cell of said mammal, said cell proliferating in said disease, said cell from a tissue; and

(b) measuring the amount of nucleic acid from said cell of said mammal that hybridizes to said molecule, an increase in the amount from said cell of said mammal relative to a control indicating a an increased likelihood of said mammal having or developing a proliferative disease.

21. The method of claim 20, wherein said method further comprises the steps of:

(a) contacting said molecule with a preparation of nucleic acid from said control, wherein said control is a cell from said tissue of a second mammal, said second mammal lacking a proliferative disease; and

(b) measuring the amount of nucleic acid from said control, an increase in the amount of said nucleic acid from said cell of said mammal that hybridizes to said molecule relative to said amount of said nucleic acid from said control indicating an increased likelihood of said mammal having or developing a proliferative disease.

22. The method of claim 20 or 21, said method further comprising the steps of:

(a) providing a pair of oligonucleotides having sequence identity to or being complementary to a region of said IAP or said NAIP nucleic acid molecule;

(b) combining said pair of oligonucleotides with said nucleic acid under conditions suitable for polymerase chain reaction-mediated nucleic acid amplification; and

(c) isolating said amplified nucleic acid or fragment thereof.

23. The method of claim 22, wherein said amplification is carried out using a reverse-transcription polymerase chain reaction.

24. The method of claim 23, wherein said reverse-transcription polymerase chain reaction is RACE.

25. The method of claim 20, 21, or 22, wherein said method provides measuring said nucleic acid having a nucleotide sequence that has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, or the nucleic acid sequence of NAIP.

26. The method of claim 20, 21, or 22, wherein said method provides measuring said nucleic acid having a nucleotide sequence that has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 3.

27. The method of claim 20, 21, or 22, wherein said method provides measuring said nucleic acid having a nucleotide sequence that has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 5.

28. The method of claim 20, 21, or 22, wherein said method provides measuring said nucleic acid having a nucleotide sequence that has about 50% or greater identity with the nucleotide sequence of SEQ ID NO: 7.

29. The method of claim 20, 21, or 22, wherein said method provides measuring said nucleic acid having a nucleotide sequence that has about 50% or greater identity with the nucleotide sequence of NAIP.

30. A method for detecting a proliferative disease or an increased likelihood of developing said disease in a mammal, said method comprising measuring the level of biological activity of an IAP polypeptide or a NAIP polypeptide in a sample of said mammal, an increase in said level of said IAP polypeptide or said NAIP polypeptide relative to a sample from a control mammal being an indication that said mammal has said disease or increased likelihood of developing said disease.

31. The method of claim 30, wherein said sample comprises a cell that is proliferating in said disease from said mammal, said cell from a tissue.

32. The method of claim 31, wherein said sample from a control mammal is from said tissue, said sample consisting of healthy cells.

33. The method of claim 32, wherein said mammal and said control mammal are the same.

34. The method of claim 30, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.

35. The method of claim 34, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

36. The method of claim 30, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

37. The method of claim 30, wherein said polypeptide is NAIP.

38. The method of claim 30, wherein said polypeptide is XIAP.

39. The method of claim 30, wherein said polypeptide is HIAP-1.

40. The method of claim 30, wherein said polypeptide is HIAP-2.

41. A method for identifying a compound enhances apoptosis in an affected cell that is proliferating in a proliferative disease, said method comprising exposing a cell that overexpresses an IAP polypeptide or a NAIP polypeptide to a candidate compound, a decrease the level of biological activity of said polypeptide indicating the presence of a compound that enhances apoptosis in said affected cell that is proliferating in said proliferative disease.

42. A method for identifying a compound that enhances apoptosis in an affected cell that is proliferating in a proliferative disease, said method comprising the steps of:

(a) providing a cell comprising a nucleic acid molecule encoding a IAP polypeptide or a nucleic acid molecule encoding a NAIP polypeptide, said nucleic acid molecule being expressed in said cell; and

(b) contacting said cell with a candidate compound and monitoring level of biological activity of said IAP polypeptide or said NAIP polypeptide in said cell, a decrease in the level of biological activity of said IAP polypeptide or said NAIP polypeptide in said cell in response to said candidate compound relative to a cell not contacted with said candidate compound indicating the presence of a compound that enhances apoptosis in said affected cell that is proliferating in said proliferative disease.

43. The method of claim 42, wherein said cell further expresses a p53 polypeptide associated with said proliferative disease.

44. The method of claim 41 or 42, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.

45. The method of claim 44, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

46. The method of claim 41 or 42, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

47. The method of claim 41 or 42, wherein said polypeptide is NAIP.

48. The method of claim 41 or 42, wherein said polypeptide is XIAP.

49. The method of claim 41 or 42, wherein said polypeptide is HIAP-1.

50. The method of claim 41 or 42, wherein said polypeptide is HIAP-2.

51. A method for determining the prognosis of a mammal diagnosed with a proliferative disease, said method comprising the steps of:

(a) isolating a sample from a tissue from said mammal; and

(b) determining whether said sample has an increased an level of biological activity of an IAP polypeptide or an NAIP polypeptide relative to a control sample, an increase in said level in said sample being an indication that said mammal has a poor prognosis.

52. The method of claim 51, wherein said sample comprises a cells that is proliferating in said proliferative disease and said control sample is from said tissue, said control sample consisting of healthy cells.

53. The method of claim 52, wherein said sample and said control sample are from said mammal.

54. The method of claim 51, wherein said sample further comprises a cell expressing a p53 polypeptide associated with said proliferative disease.

55. The method of claim 51, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.

56. The method of claim 55, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

57. The method of claim 51, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

58. The method of claim 51, wherein said polypeptide is NAIP.

59. The method of claim 51, wherein said polypeptide is XIAP.

60. The method of claim 51, wherein said polypeptide is HIAP-1.

61. The method of claim 51, wherein said polypeptide is HIAP-2.

62. The method of claim 51, wherein said level is assayed by measuring the amount of IAP peptide of less than 64 kDa present in said sample.

63. A method for determining the prognosis of a mammal diagnosed with a proliferative disease, said method comprising the steps of:

(a) isolating a sample from said mammal, said sample having a nuclear fraction; and

(b) measuring the amount of a polypeptide that is recognized by an antibody that specifically binds an IAP polypeptide or an antibody that specifically binds an NAIP polypeptide in said nuclear fraction of said sample relative an amount from a control sample, an increase in said amount from said sample being an indication that said mammal has a poor prognosis.

64. The method of claim 63, wherein said sample is from a tissue of said mammal, said sample comprising a cell that is proliferating in said proliferative disease, and said control sample is from said tissue, said control sample consisting of healthy cells.

65. The method of claim 64, wherein said sample and said control sample are from said mammal.

66. The method of claim 63, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.

67. The method of claim 66, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

68. The method of claim 63, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

69. The method of claim 63, wherein said polypeptide is NAIP.

70. The method of claim 63, wherein said polypeptide is XIAP.

71. The method of claim 63, wherein said polypeptide is HIAP-1.

72. The method of claim 63, wherein said polypeptide is HIAP-2.

73. The method of claim 63, wherein said amount is measured by immunological methods.

74. A method for treating a mammal diagnosed as having a proliferative disease, said method comprising the steps of:

(a) measuring the amount of an IAP or NAIP polypeptide in a first sample from a tissue from said mammal, said first sample comprising a cell that is proliferating in said proliferative disease;

(b) measuring the amount of said polypeptide in a second sample from said tissue, said second sample consisting of healthy cells;

(c) detecting an increase in the amount of said polypeptide in said first sample to the amount of said polypeptide in said second sample; and

(d) treating said mammal with a compound that decreases the biological activity of said polypeptide.

75. The method of claim 74, wherein said first sample and said second sample are from said mammal.

76. The method of claim 74, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.

77. The method of claim 76, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

78. The method of claim 74, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

79. The method of claim 74, wherein said polypeptide is NAIP.

80. The method of claim 74, wherein said polypeptide is XIAP.

81. The method of claim 74, wherein said polypeptide is HIAP-1.

82. The method of claim 74, wherein said polypeptide is HIAP-2.

83. Use of a compound that decreases the biological activity an IAP polypeptide or a NAIP polypeptide for the manufacture of a medicament for the enhancement of apoptosis.

84. The use of claim 83, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.

85. The use of claim 84, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

86. The use of claim 83, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

87. The use of claim 83, wherein said polypeptide is NAIP.

88. The use of claim 83, wherein said polypeptide is XIAP.

89. The use of claim 83, wherein said polypeptide is HIAP-1.

90. The use of claim 83, wherein said polypeptide is HIAP-2.

91. A kit for diagnosing a mammal for the presence of a proliferative disease or an increased likelihood of developing a proliferative disease, said kit compromising an oligonucleotide that hybridizes to a nucleic acid sequence that encodes an IAP polypeptide or a NAIP polypeptide.

92. The kit of claim 91, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

93. The kit of claim 91, wherein said polypeptide is NAIP.

94. The kit of claim 91, wherein said polypeptide is XIAP.

95. The kit of claim 91, wherein said polypeptide is HIAP-1.

96. The kit of claim 91, wherein said polypeptide is HIAP-2.

97. A transgenic mammal, said mammal having an elevated level of biological activity of an IAP polypeptide or a NAIP polypeptide.

98. The transgenic mammal of claim 97, wherein said biological activity is the level of expression of said polypeptide; wherein said biological activity is the level of expression of an mRNA molecule encoding said polypeptide; or wherein said biological activity is an apoptosis-inhibiting activity.

99. The transgenic mammal of claim 98, wherein said level of expression is measured by assaying the amount of said polypeptide present in said cell.

100. The transgenic mammal of claim 97, wherein said polypeptide is selected from the group consisting of HIAP-1, m-HIAP-1, HIAP-2, m-HIAP-2, XIAP, and m-XIAP.

101. The transgenic mammal of claim 97, wherein said polypeptide is NAIP.

102. The transgenic mammal of claim 97, wherein said polypeptide is XIAP.

103. The transgenic mammal of claim 97, wherein said polypeptide is HIAP-1.

104. The transgenic mammal of claim 97, wherein said polypeptide is HIAP-2.

```
SEQ ID 3—1   GAAAAGGTGGACAAGTCCTATTTTCAAGAGAAGATGACTTTTAACAGTTTTGAAGGATCT 60
SEQ ID 4—1                                    M  T  F  N  S  F  E  G  S   9

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     121   TTAAAAACTTTTGCTAATTTTCCAAGTGGTAGTCCTGTTTCAGCATCAACACTGGCACGA 180
      30   L  K  T  F  A  N  F  P  S  G  S  P  V  S  A  S  T  L  A  R   49

     181   GCAGGGTTTCTTTATACTGGTGAAGGAGATACCGTGCGGTGCTTTAGTTGTCATGCAGCT 240
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     241   GTAGATAGATGGCAATATGGAGACTCAGCAGTTGGAAGACACAGGAAAGTATCCCCAAAT 300
      70   V  D  R  W  Q  Y  G  D  S  A  V  G  R  H  R  K  V  S  P  N   89

     301   TGCAGATTTATCAACGGCTTTTATCTTGAAAATAGTGCCACGCAGTCTACAAATTCTGGT 360
      90   C  R  F  I  N  G  F  Y  L  E  N  S  A  T  Q  S  T  N  S  G  109

     361   ATCCAGAATGGTCAGTACAAAGTTGAAAACTATCTGGGAAGCAGAGATCATTTTGCCTTA 420
     110   I  Q  N  G  Q  Y  K  V  E  N  Y  L  G  S  R  D  H  F  A  L  129

     421   GACAGGCCATCTGAGACACATGCAGACTATCTTTTGAGAACTGGGCAGGTTGTAGATATA 480
     130   D  R  P  S  E  T  H  A  D  Y  L  L  R  T  G  Q  V  V  D  I  149

     481   TCAGACACCATATACCCGAGGAACCCTGCCATGTATaGTGAAGAAGCTAGATTAAAGTCC 540
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     601   TACTACACAGGTATTGGTGACCAAGTGCAGTGCTTTTGTTGTGGTGGAAAACTGAAAAAT 660
     190   Y  Y  T  G  I  G  D  Q  V  Q  C  F  C  C  G  G  K  L  K  N  209

     661   TGGGAACCTTGTGATCGTGCCTGGTCAGAACACAGGCGACACTTTCCTAATTGCTTCTTT 720
     210   W  E  P  C  D  R  A  W  S  E  H  R  R  H  F  P  N  C  F  F  229

     721   GTTTTGGGCCGGAATCTTAATATTCGAAGTGAATCTGATGCTGTGAGTTCTGATAGGAAT 780
     230   V  L  G  R  N  L  N  I  R  S  E  S  D  A  V  S  S  D  R  N  249

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     250   F  P  N  S  T  N  L  P  R  N  P  S  M  A  D  Y  E  A  R  I  269

     841   TTTACTTTTGGGACATGGATATACTCAGTTAACAAGGAGCAGCTTGCAAGAGCTGGATTT 900
     270   F  T  F  G  T  W  I  Y  S  V  N  K  E  Q  L  A  R  A  G  F  289
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     901   TATGCTTTAGGTGAAGGTGATAAAGTAAAGTGCTTTCACTGTGGAGGAGGGCTAACTGAT 960
     290   Y  A  L  G  E  G  D  K  V  K  C  F  H  C  G  G  G  L  T  D  309
                                                   2  3
     961   TGGAAGCCCAGTGAAGACCCTTGGGAACAACATGCTAAATGGTATCCAGGGTGCAAATAT 1020
     310   W  K  P  S  E  D  P  W  E  Q  H  A  K  W  Y  P  G  C  K  Y  329

    1021   CTGTTAGAACAGAAGGGACAAGAATATATAAACAATATTCATTTAACTCATTCACTTGAG 1080
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```

Fig. 1

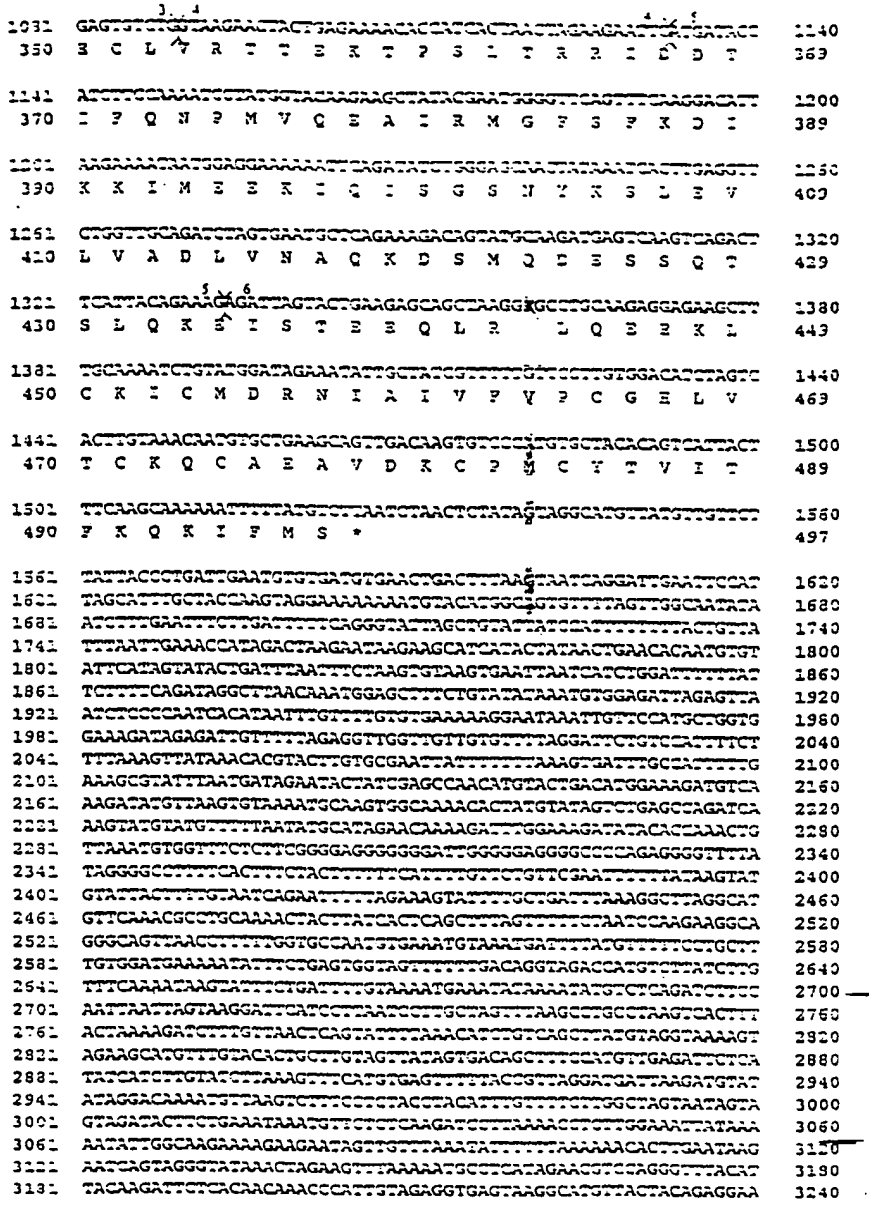

Fig 1

Sheet 2 of 3

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3061 AATATTGGCAAGAAAAGAAGAATAGTTGTTTAAATATTTTTTAAAAAACACTTGAATAAG 3120
3121 AATCAGTAGGGTATAAACTAGAAGTTTAAAAATGCCTCATAGAACGTCCAGGGTTTACAT 3180
3181 TACAAGATTCTCACAACAAACCCATTGTAGAGGTGAGTAAGGCATGTTACTACAGAGGAA 3240
3241 AGTTTGAGAGTAAAACTGTAAAAAATTATATTTTTGTTGTACTTTCTAAGAGAAAGAGTA 3300
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3361 GCAAATTTATTTTATTTATTTAATTTTCTTTTTGAGATGGAGTCTTGCTTGTCACCCAGG 3420
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4921 ATGAAATAAAATGGGGCTGGGCTCAGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGG 4980
4981 CTGAGGCAGGTGGATCACGAGGTCAGGAGATCGAGACCATCCTGGCTAACACGGTGAAAC 5040
5041 CCCGTCTCTACTAAAAAACAGAAAATTAGCCGGGCGTGGTGGCGGGCGCCTGTAGTCCCA 5100
5101 GCTACTCGGGAGGCTGAGGCAGGAGAATGGTGTGAACCCGGGAGGCAGAGCTTGCAGTGA 5160
5161 GCCGAGATCTCGCCACTGCACTCCAGCCTGGGCAACAGAGCAAGACTCTGTCTCAAAAAA 5220
5221 AAAAAAAAAAAG  5232
```

Fig. 1 (cont.)

SEQ. ID 5— 1 TTGCTCTGTCACCCAGTTTGGAGTGCAGTTATGCAGTCTCACACTGCAAGCTCTGCCTCA 60
61 TGGGCTCAAGTGAACCTCCTGCCTCAGCCTCTCAAGTAGCTGGGACCACAGGCAGGTGCC 120
121 ACCATGTCTGGCTAATTTTTGAGTTTCTTTGTAGAGATGGTGTTTTGCCAAGTCACCCAG 180
181 TTTGAGGCTGGTCTCAAACACCTGGGCTCAAGCAATCCATCTACCTCAGCCTCCCAAAGT 240
241 GCTGGGATTACAGGAGTGAGCCATGGCATGAGGCCTTGTGGGGTGTCTCTTTTAAATGAA 300
301 AGCATACTCTGTTTACGTATTTGATATGAAGGAATATCCTTCCTTTCCACAAAGACAAAA 360
361 ATTATCCTATTTTTCTCAAAACATATGTCCTTTTTCTCTACTTTTCATTTTTGTTACTTT 420
421 TGATGGACACATGTGTTACATTGATTTCACTTTCTCATAATTCTGCTGTAAGAAAAACAA 480
481 TAGTGCCAGTTCAATGACAAATAGCAACAGTCTGTTATTGCTAGACTGTTACTGTTAGTG 540
541 GAGACTACCAGAACAGTCAGTCCCAGTGTCAGGGAATCAAAGAGAACATGTTCCCTCTCT 600
601 AAAGGGCACAGCTGCTGCTCAGCTTTAGCTGATTGCTGCCCTGCAGGACTATAGGCCCAG 660
661 TGTTGCTAGATCTTTTGATGTTTCAAGAGAAGCTTGGAATCTAGAATGTGATGGGAAGTC 720
721 TCTTACATTTAAACATGTTGGCAATTAATGGTAAGATTTAAAAATACTGTGGTCCAAGAA 780
781 AAAAATGGATTTGGAAACTGGATTAAATTCAAATGAGGCATGCAGATTAATCTACAGCAT 840
841 GGTACAATGTGAATTTTCTGGTTTCTTTAATTGCACTGTAATTAGGTAAGATGTTAGCTT 900
901 TGGGGAAGCTAAGTGCAGAGTATGCAGAAACTATTATTTTTGTAAGTTTTCTCTAAGTAT 960
961 AAATAAATTTCAAAATAAAAATAAAAACTTAGTAAAGAACTATAATGCAATTCTATGTAA 1020
1021 GCCAAACATAATATGTCTTCCAGTTTGAAACCTCTGGGTTTTATTTTATTTTATTTTATT 1080
1081 TTTGAGACAGAGTCTTGCTGTGTCACCCAGGCTGGAGTGTAGTGGCACTATTTCGGCCCA 1140
1141 CTGCAACCTCCACCTCCCAGGCTCAAATGATTCTCCTGCCTCAGCCTCCGGAGTAGCTGG 1200
1201 GATTACAGGCGCGTACCACCACACCCAGCTAATTTTTGTATTTTTAGTAGAGATGGGGTT 1260
1261 TCACCATTTTGGCCAGGCTGGTTTTGAACTCCTGACCTCAAGTGATCCACTTGTCTTGGC 1320
1321 CTCCCAAAATGCTGGGATTACAGGCGTGAGCCACTGCACCAGGCAGAGGCCTCTGTTTTT 1380
1381 TATCTCTTTTTGGCCTCTACAGTGCCTAGTAAAGCACCTGATACATGGTAAACGATCAGT 1440
1441 AATTACTAGTACTCTATTTTGGAGAAAATGATTTTTTAAAAAGTCATTGTGTTCCATCCA 1500
1501 TGAGTCGTTTGAGTTTTAAAACTGTCTTTTTGTTTGTTTTTGAACAGGTTTACAAAGGAG 1560
1561 GAAAACGACTTCTTCTAGATTTTTTTTTCAGTTTCTTCTATAAATCAAAACATCTCAAAA 1620
1621 TGGAGACCTAAAATCCTTAAAGGGACTTAGTCTAATCTCGGGAGGTAGTTTTGTGCATGG 1680
1681 GTAAACAAATTAAGTATTAACTGGTGTTTTACTATCCAAAGAATGCTAATTTTATAAACA 1740
1741 TGATCGAGTTATATAAGGTATACCATAATGAGTTTGATTTTGAATTTGATTTGTGGAAAT 1800
1801 AAAGGAAAAGTGATTCTAGCTGGGGCATATTGTTAAAGCATTTTTTTCAGAGTTGGCCAG 1860
1861 GCAGTCTCCTACTGGCACATTCTCCCATTATGTAGAATAGAAATAGTACCTGTGTTTGGG 1920
1921 AAAGATTTTAAAATGAGTGACAGTTATTTGGAACAAAGAGCTAATAATCAATCCACTGCA 1980
1981 AATTAAAGAAACATGCAGATGAAAGTTTTGACACATTAAAATACTTCTACAGTGACAAAG 2040
2041 AAAAATCAAGAACAAAGCTTTTTGATATGTGCAACAAATTTAGAGGAAGTAAAAAGATAA 2100
2101 ATGTGATGATTGGTCAAGAAATTATCCAGTTATTTACAAGGCCACTGATATTTTAAACGT 2160
2161 CCAAAAGTTTGTTTAAATGGGCTGTTACCGCTGAGAATGATGAGGATGAGAATGATGGTT 2220
2221 GAAGGTTACATTTTAGGAAATGAAGAAACTTAGAAAATTAATATAAAGACAGTGATGAAT 2280
2281 ACAAAGAAGATTTTTATAACAATGTGTAAAATTTTTGGCCAGGGAAAGGAATATTGAAGT 2340
2341 TAGATACAATTACTTACCTTTGAGGGAAATAATTGTTGGTAATGAGATGTGATGTTTCTC 2400
2401 CTGCCACCTGGAAACAAAGCATTGAAGTCTGCAGTTGAAAAGCCCAACGTCTGTGAGATC 2460
2461 CAGGAAACCATGCTTGCAAACCACTGGTAAAAAAAAAAAAAAAAAAAAAAAAAGCCACAG 2520
2521 TGACTTGCTTATTGGTCATTGCTAGTATTATCGACTCAGAACCTCTTTACTAATGGCTAG 2580
2581 TAAATCATAATTGAGAAATTCTGAATTTTGACAAGGTCTCTGCTGTTGAAATGGTAAATT 2640
2641 TATTATTTTTTTTTGTCATGATAAATTCTGGTTCAAGGTATGCTATCCATGAAATAATTTC 2700
2701 TGACCAAAACTAAATTGATGCAATTTGATTATCCATCTTAGCCTACAGATGGCATCTGGT 2760
2761 AACTTTTGACTGTTTTAAAAAATAAATCCACTATCAGAGTAGATTTGATGTTGGCTTCAG 2820
2821 AAACATTTAGAAAAACAAAAGTTCAAAAATGTTTTCAGGAGGTGATAAGTTGAATAACTC 2880
2881 TACAATGTTAGTTCTTTGAGGGGGACARAAAATTTAAAATCTTTGAAAGGTCTTATTTTA 2940
2941 CAGCCATATCTAAATTATCTTAAGAAAATTTTTAACAAAGGGAATGAAATATATATCATG 3000
3001 ATTCTGTTTTTCCAAAAGTAACCTGAATATAGCAATGAAGTTCAGTTTTGTTATTGGTAG 3060
3061 TTTGGGCAGAGTCTCTTTTTGCAGCACCTGTTGTCTACCATAATTACAGAGGACATTTCC 3120
3121 ATGTTCTAGCCAAGTATACTATTAGAATAAARAAACTTAACATTGAGTTGCTTCAACAGC 3180

Fig. 2

```
3181 ATGAAACTGAGTCCAAAAGACCAAATGAACAAACACATTAATCTCTGATTATTTATTTTA 3240
3241 AATAGAATATTTAATTGTGTAAGATCTAATAGTATCATTATACTTAAGCAATCATATTCC 3300
3301 TGATGATCTATGGGAAATAACTATTATTTAATTAATATTGAAACCAGGTTTTAAGATGTG 3360
3361 TTAGCCAGTCCTGTTACTAGTAAATCTCTTTATTTGGAGAGAAATTTTAGATTGTTTTGT 3420
3421 TCTCCTTATTAGAAGGATTGTAGAAAGAAAAAAATGACTAATTGGAGAAAAATTGGGGAT 3480
3481 ATATCATATTTCACTGAATTCAAAATGTCTTCAGTTGTAAATCTTACCATTATTTTACGT 3540
3541 ACCTCTAAGAAATAAAAGTGCTTCTAATTAAAATATGATGTCATTAATTATGAAATACTT 3600
3601 CTTGATAACAGAAGTTTTAAAATAGCCATCTTAGAATCAGTGAAATATGGTAATGTATTA 3660
3661 TTTTCCTCCTTTGAGTNAGGTCTTGTGCTTTTTNTTCCTGGCCACTAAATNTCACCATNT 3720
3721 CCAANAAGCAAANTAAACCTATTCTGAATATTTTTGCTGTGAAACACTTGNCAGCAGAGC 3780
3781 TTTCCCNCCATGNNAGAAGCTTCATGAGTCACACATTACATCTTTGGGTTGATTGAATGC 3840
3841 CACTGAAACATTTCTAGTAGCCTGGAGNAGTTGACCTACCTGTGGAGATGCCTGCCATTA 3900
3901 AATGGCATCCTGATGGCTTAATACACATCACTCTTCTGTGNAGGGTTTTAATTTTCAACA 3960
3961 CAGCTTACTCTGTAGCATCATGTTTACATTGTATGTATAAAGATTATACNAAGGTGCAAT 4020
4021 TGTGTATTTCTTCCTTAAAATGTATCAGTATAGGATTTAGAATCTCCATGTTGAAACTCT 4080
4081 AAATGCATAGAAATAAAAATAATAAAAAATTTTTCATTTTGGCTTTTCAGCCTAGTATTA 4140
4141 AAACTGATAAAAGCAAAGCCATGCACAAAACTACCTCCCTAGAGAAAGGCTAGTCCCTTT 4200
4201 TCTTCCCCATTCATTTCATTATGAACATAGTAGAAAACAGCATATTCTTATCAAATTTGA 4260
SEQ. ID 6—1                    M  N  I  V  E  N  S  I  F  L  S  N  L  M 14

4261 TGAAAAGCGCCAACACGTTTGAACTGAAATACGACTTGTCATGTGAACTGTACCGAATGT 4320
  15   K  S  A  N  T  F  E  L  K  Y  D  L  S  C  E  L  Y  R  M  S 34

4321 CTACGTATTCCACTTTTCCTGCTGGGGTTCCTGTCTCAGAAAGGAGTCTTGCTCGTGCTG 4380
  35   T  Y  S  T  F  P  A  G  V  P  V  S  E  R  S  L  A  R  A  G 54

4381 GTTTCTATTACACTGGTGTGAATGACAAGGTCAAATGCTTCTGTTGTGGCCTGATGCTGG 4440
  55   F  Y  Y  T  G  V  N  D  K  V  K  C  F  C  C  G  L  M  L  D 74

4441 ATAACTGGAAAAGAGGAGACAGTCCTACTGAAAAGCATAAAAAGTTGTATCCTAGCTGCA 4500
  75   N  W  K  R  G  D  S  P  T  E  K  H  K  K  L  Y  P  S  C  R 94

4501 GATTCGTTCAGAGTCTAAATTCCGTTAACAACTTGGAAGCTACCTCTCAGCCTACTTTTC 4560
  95   F  V  Q  S  L  N  S  V  N  N  L  E  A  T  S  Q  P  T  F  P 114

4561 CTTCTTCAGTAACACATTCCACACACTCATTACTTCCGGGTACAGAAAACAGTGGATATT 4620
 115   S  S  V  T  H  S  T  H  S  L  L  P  G  T  E  N  S  G  Y  F 134

4621 TCCGTGGCTCTTATTCAAACTCTCCATCAAATCCTGTAAACTCCAGAGCAAATCAAGAAT 4680
 135   R  G  S  Y  S  N  S  P  S  N  P  V  N  S  R  A  N  Q  E  F 154

4681 TTTCTGCCTTGATGAGAAGTTCCTACCCCTGTCCAATGAATAACGAAAATGCCAGATTAC 4740
 155   S  A  L  M  R  S  S  Y  P  C  P  M  N  N  E  N  A  R  L  L 174

4741 TTACTTTTCAGACATGGCCATTGACTTTTCTGTCGCCAACAGATCTGGCACGAGCAGGCT 4800
 175   T  F  Q  T  W  P  L  T  F  L  S  P  T  D  L  A  R  A  G  F 194

4801 TTTACTACATAGGACCTGGAGACAGAGTGGCTTGCTTTGCCTGTGGTGGAAAATTGAGCA 4860
 195   Y  Y  I  G  P  G  D  R  V  A  C  F  A  C  G  G  K  L  S  N 214

4861 ATTGGGAACCGAAGGATAATGCTATGTCAGAACACCTGAGACATTTTCCCAAATGCCCAT 4920
 215   W  E  P  K  D  N  A  M  S  E  H  L  R  H  F  P  K  C  P  F 234
```

Fig. 2 (cont.)

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4921 TTATAGAAAATCAGCTTCAAGACACTTCAAGATACACAGTTTCTAATCTGAGCATGCAGA 4980
 235   I  E  N  Q  L  Q  D  T  S  R  Y  T  V  S  N  L  S  M  Q  T 254

4981 CACATGCAGCCCGCTTTAAAACATTCTTTAACTGGCCCTCTAGTGTTCTAGTTAATCCTG 5040
 255   H  A  A  R  F  K  T  F  F  N  W  P  S  S  V  L  V  N  P  E 274
                                      1  2
5041 AGCAGCTTGCAAGTGCGGGTTTTTATTATGTGGGTAACAGTGATGATGTCAAATGCTTTT 5100
 275   Q  L  A  S  A  G  F  Y  Y  V  G  N  S  D  D  V  K  C  F  C 294

5101 GCTGTGATGGTGGACTCAGGTGTTGGGAATCTGGAGATGATCCATGGGTTCAACATGCCA 5160
 295   C  D  G  G  L  R  C  W  E  S  G  D  D  P  W  V  Q  H  A  K 314
             2  3
5161 AGTGGTTTCCAAGGTGTGAGTACTTGATAAGAATTAAAGGACAGGAGTTCATCCGTCAAG 5220
 315   W  F  P  R  C  E  Y  L  I  R  I  K  G  Q  E  F  I  R  Q  V 334
                                      3  4
5221 TTCAAGCCAGTTACCCTCATCTACTTGAACAGCTGCTATCCACATCAGACAGCCCAGGAG 5280
 335   Q  A  S  Y  P  H  L  L  E  Q  L  L  S  T  S  D  S  P  G  D 354
                   4  5
5281 ATGAAAATGCAGAGTCATCAATTATCCATTTTGAACCTGGAGAAGACCATTCAGAAGATG 5340
 355   E  N  A  E  S  S  I  I  H  F  E  P  G  E  D  H  S  E  D  A 374

5341 CAATCATGATGAATACTCCTGTGATTAATGCTGCCGTGGAAATGGGCTTTAGTAGAAGCC 5400
 375   I  M  M  N  T  P  V  I  N  A  A  V  E  M  G  F  S  R  S  L 394

5401 TGGTAAAACAGACAGTTCAGAGAAAAATCCTAGCAACTGGAGAGAATTATAGACTAGTCA 5460
 395   V  K  Q  T  V  Q  R  K  I  L  A  T  G  E  N  Y  R  L  V  N 414

5461 ATGATCTTGTGTTAGACTTACTCAATGCAGAAGATGAAATAAGGGAAGAGGAGAGAGAAA 5520
 415   D  L  V  L  D  L  L  N  A  E  D  E  I  R  E  E  E  R  E  R 434
                      5  6
5521 GAGCAACTGAGGAAAAAGAATCAAATGATTTATTATTAATCCGGAAGAATAGAATGGCAC 5580
 435   A  T  E  E  K  E  S  N  D  L  L  L  I  R  K  N  R  M  A  L 454

5581 TTTTTCAACATTTGACTTGTGTAATTCCAATCCTGGATAGTCTACTAACTGCCGGAATTA 5640
 455   F  Q  H  L  T  C  V  I  P  I  L  D  S  L  L  T  A  G  I  I 474

5641 TTAATGAACAAGAACATGATGTTATTAAACAGAAGACACAGACGTCTTTACAAGCAAGAG 5700
 475   N  E  Q  E  H  D  V  I  K  Q  K  T  Q  T  S  L  Q  A  R  E 494

5701 AACTGATTGATACGATTTTAGTAAAAGGAAATATTGCAGCCACTGTATTCAGAAACTCTC 5760
 495   L  I  D  T  I  L  V  K  G  N  I  A  A  T  V  F  R  N  S  L 514
                                                  6  7
5761 TGCAAGAAGCTGAAGCTGTGTTATATGAGCATTTATTTGTGCAACAGGACATAAAATATA 5820
 515   Q  E  A  E  A  V  L  Y  E  H  L  F  V  Q  Q  D  I  K  Y  I 534
                         7  8
5821 TTCCCACAGAAGATGTTTCAGATCTACCAGTGGAAGAACAATTGCGGAGACTACAAGAAG 5880
 535   P  T  E  D  V  S  D  L  P  V  E  E  Q  L  R  R  L  Q  E  E 554

5881 AAAGAACATGTAAAGTGTGTATGGACAAAGAAGTGTCCATAGTGTTTATTCCTTGTGGTC 5940
 555   R  T  C  K  V  C  M  D  K  E  V  S  I  V  F  I  P  C  G  H 574

5941 ATCTAGTAGTATGCAAAGATTGTGCTCCTTCTTTAAGAAAGTGTCCTATTTGTAGGAGTA 6000
 575   L  V  V  C  K  D  C  A  P  S  L  R  K  C  P  I  C  R  S  T 594
```

Fig. 2 (cont.)

```
6001 CAATCAAGGGTACAGTTCGTACATTTCTTTCATGAAGAAGAACCAAAACATCGTCTAAAC 6060
 595   I  K  G  T  V  R  T  F  L  S  *                            604

6061 TTTAGAATTAATTTATTAAATGTATTATAACTTTAACTTTTATCCTAATTTGGTTTCCTT 6120
6121 AAAATTTTTATTTATTTACAACTCAAAAAACATTGTTTTGTGTAACATATTTATATATGT 6180
6181 ATCTAAACCATATGAACATATATTTTTTAGAAACTAAGAGAATGATAGGCTTTTGTTCTT 6240
6241 ATGAACGAAAAAGAGGTAGCACTACAAACACAATATTCAATCAAAATTTCAGCATTATTG 6300
6301 AAATTGTAAGTGAAGTAAAACTTAAGATATTTGAGTTAACCTTTAAGAATTTTAAATATT 6360
6361 TTGGCATTGTACTAATACCGGGAACATGAAGCCAGGTGTGGTGGTATGTGCCTGTAGTCC 6420
6421 CAGGCTGAGGCAAGAGAATTACTTGAGCCCAGGAGTTTGAATCCATCCTGGGCAGCATAC 6480
6481 TGAGACCCTGCCTTTAAAAACAAACAGAACAAAAACAAAACACCAGGGACACATTTCTCT 6540
6541 GTCTTTTTTGATCAGTGTCCTATACATCGAAGGTGTGCATATATGTTGAATCACATTTTA 6600
6601 GGGACATGGTGTTTTTATAAAGAATTCTGTGAGAAAAAATTTAATAAAGCAACCAAAAAA 6660
6661 AAAAAAAAA 6669
```

Fig. 2 (cont.)

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                                  1 2
SEQ.ID7— 1 GAGCGCCCGGGCTGATCCGAGCCGAGCGGGCCGTATCTCCTTGTCGGCGCCGCTGATTCC 60
        61 CGGCTCTGCGGAGGCCTCTAGGCAGCCGCGCAGCTTCCGTGTTTGCTGCGCCCGCACTGC 120
          2 3
       121 GATTTACAACCCTGAAGAATCTCCCTATCCCTATTTTGTCCCCCTGCAGTAATAAATCCC 180
       181 ATTATGGAGATCTCGAAACTTTATAAAGGGATATAGTTTGAATTCTATGGAGTGTAATTT 240
       241 TGTGTATGAATTATATTTTTAAAACATTGAAGAGTTTTCAGAAAGAAGGCTAGTAGAGTT 300
       301 GATTACTGATACTTTATGCTAAGCAGTACTTTTTTGGTAGTACAATATTTTGTTAGGCGT 360
       361 TTCTGATAACACTAGAAAGGACAAGTTTTATCTTGTGATAAATTGATTAATGTTTACAAC 420
       421 ATGACTGATAATTATAGCTGAATAGTCCTTAAATGATGAACAGGTTATTTAGTTTTTAAA 480
       481 TGCAGTGTAAAAAGTGTGCTGTGGAAATTTTATGGCTAACTAAGTTTATGGAGAAAATAC 540
       541 CTTCAGTTGATCAAGAATAATAGTGGTATACAAAGTTAGGAAGAAAGTCAACATGATGCT 600
       601 GCAGGAAATGGAAACAAATACAAATGATATTTAACAAAGATAGAGTTTACAGTTTTTGAA 660
       661 CTTTAAGCCAAATTCATTTGACATCAAGCACTATAGCAGGCACAGGTTCAACAAAGCTTG 720
       721 TGGGTATTGACTTCCCCCAAAAGTTGTCAGCTGAAGTAATTTAGCCCACTTAAGTAAATA 780
       781 CTATGATGATAAGCTGTGTGAACTTAGCTTTTAAATAGTGTGACCATATGAAGGTTTTAA 840
       841 TTACTTTTGTTTATTGGAATAAAATGAGATTTTTTGGGTTGTCATGTTAAAGTGCTTATA 900
       901 GGGAAAGAAGCCTGCATATAATTTTTTACCTTGTGGCATAATCAGTAATTGGTCTGTTAT 960
       961 TCAGGCTTCATAGCTTGTAACCARATATAAATAAAAGGCATAATTTAGGTATTCTATAGT 1020
      1021 TGCTTAGAATTTTGTTAATATAAATCTCTGTGAAAAATCAAGGAGTTTTAATATTTTCAG 1080
      1081 AAGTGCATCCACCTTTCAGGGCTTTAAGTTAGTATTAACTCAAGATTATGAACAAATAGC 1140
      1141 ACTTAGGTTACCTGAAAGAGTTACTACAACCCCAAAGAGTTGTGTTCTAAGTAGTATCTT 1200
      1201 GGTAATTCAGAGAGATACTCATCCTACCTGAATATAAACTGAGATAAATCCAGTAAAGAA 1260
      1261 AGTGTAGTAAATTCTACATAAGAGTCTATCATTGATTTCTTTTTGTGGTAAAAATCTTAG 1320
      1321 TTCATGTGAAGAAATTTCATGTGAATGTTTTAGCTATCAAACAGTACTGTCACCTACTCA 1380
                                                                M 1
      1381 TGCACAAAACTGCCTCCCAAAGACTTTTCCCAGGTCCCTCGTATCAAAACATTAAGAGTA 1440
SEQ.ID8— 2   H  K  T  A  S  Q  R  L  F  P  G  P  S  Y  Q  N  I  K  S  I 21
      1441 TAATGGAAGATAGCACGATCTTGTCAGATTGGACAAACAGCAACAAACAAAAAATGAAGT 1500
        22   M  E  D  S  T  I  L  S  D  W  T  N  S  N  K  Q  K  M  K  Y 41
      1501 ATGACTTTTCCTGTGAACTCTACAGAATGTCTACATATTCAACTTTCCCCGCCGGGGTGC 1560
        42   D  F  S  C  E  L  Y  R  M  S  T  Y  S  T  F  P  A  G  V  P 61
      1561 CTGTCTCAGAAAGGAGTCTTGCTCGTGCTGGTTTTTATTATACTGGTGTGAATGACAAGG 1620
        62   V  S  E  R  S  L  A  R  A  G  F  Y  Y  T  G  V  N  D  K  V 81
      1621 TCAAATGCTTCTGTTGTGGCCTGATGCTGGATAACTGGAAACTAGGAGACAGTCCTATTC 1680
        82   K  C  F  C  C  G  L  M  L  D  N  W  K  L  G  D  S  P  I  Q 101
      1681 AAAAGCATAAACAGCTATATCCTAGCTGTAGCTTTATTCAGAATCTGGTTTCAGCTAGTC 1740
       102   K  H  K  Q  L  Y  P  S  C  S  F  I  Q  N  L  V  S  A  S  L 121
      1741 TGGGATCCACCTCTAAGAATACGTCTCCAATGAGAAACAGTTTTGCACATTCATTATCTC 1800
       122   G  S  T  S  K  N  T  S  P  M  R  N  S  F  A  H  S  L  S  P 141
      1801 CCACCTTGGAACATAGTAGCTTGTTCAGTGGTTCTTACTCCAGCCTTTCTCCAAACCCTC 1860
       142   T  L  E  H  S  S  L  F  S  G  S  Y  S  S  L  S  P  N  P  L 161
      1861 TTAATTCTAGAGCAGTTGAAGACATCTCTTCATCGAGGACTAACCCCTACAGTTATGCAA 1920
       162   N  S  R  A  V  E  D  I  S  S  S  R  T  N  P  Y  S  Y  A  M 181
      1921 TGAGTACTGAAGAAGCCAGATTTCTTACCTACCATATGTGGCCATTAACTTTTTTGTCAC 1980
       182   S  T  E  E  A  R  F  L  T  Y  H  M  W  P  L  T  F  L  S  P 201
```

Fig. 3

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1981 CATCAGAATTGGCAAGAGCTGGTTTTTATTATATAGGACCTGGAGATAGGGTAGCCTGCT 2040
 202   S  E  L  A  R  A  G  F  Y  Y  I  G  P  G  D  R  V  A  C  F 221

2041 TTGCCTGTGGTGGGAAGCTCAGTAACTGGGAACCAAAGGATGATGCTATGTCAGAACACC 2100
 222   A  C  G  G  K  L  S  N  W  E  P  K  D  D  A  M  S  E  H  R 241

2101 GGAGGCATTTTCCCAACTGTCCATTTTTGGAAAATTCTCTAGAAACTCTGAGGTTTAGCA 2160
 242   R  H  F  P  N  C  P  F  L  E  N  S  L  E  T  L  R  F  S  I 261

2161 TTTCAAATCTGAGCATGCAGACACATGCAGCTCGAATGAGAACATTTATGTACTGGCCAT 2220
 262   S  N  L  S  M  Q  T  H  A  A  R  M  R  T  F  M  Y  W  P  S 281
                                                    3  4
2221 CTAGTGTTCCAGTTCAGCCTGAGCAGCTTGCAAGTGCTGGTTTTTATTATGTGGGTCGCA 2280
 282   S  V  P  V  Q  P  E  Q  L  A  S  A  G  F  Y  Y  V  G  R  N 301

2281 ATGATGATGTCAAATGCTTTTGTTGTGATGGTGGCTTGAGGTGTTGGGAATCTGGAGATG 2340
 302   D  D  V  K  C  F  C  C  D  G  G  L  R  C  W  E  S  G  D  D 321
                                  4  5
2341 ATCCATGGGTAGAACATGCCAAGTGGTTTCCAAGGTGTGAGTTCTTGATACGAATGAAAG 2400
 322   P  W  V  E  H  A  K  W  F  P  R  C  E  F  L  I  R  M  K  G 341
                                                       5  6
2401 GCCAAGAGTTTGTTGATGAGATTCAAGGTAGATATCCTCATCTTCTTGAACAGCTGTTGT 2460
 342   Q  E  F  V  D  E  I  Q  G  R  Y  P  H  L  L  E  Q  L  L  S 361
                                           6  7
2461 CAACTTCAGATACCACTGGAGAAGAAAATGCTGACCCACCAATTATTCATTTTGGACCTG 2520
 362   T  S  D  T  T  G  E  E  N  A  D  P  P  I  I  H  F  G  P  G 381

2521 GAGAAAGTTCTTCAGAAGATGCTGTCATGATGAATACACCTGTGGTTAAATCTGCCTTGG 2580
 382   E  S  S  S  E  D  A  V  M  M  N  T  P  V  V  K  S  A  L  E 401

2581 AAATGGGCTTTAATAGAGACCTGGTGAAACAAACAGTTCAAAGTAAAATCCTGACAACTG 2640
 402   M  G  F  N  R  D  L  V  K  Q  T  V  Q  S  K  I  L  T  T  G 421

2641 GAGAGAACTATAAAACAGTTAATGATATTGTGTCAGCACTTCTTAATGCTGAAGATGAAA 2700
 422   E  N  Y  K  T  V  N  D  I  V  S  A  L  L  N  A  E  D  E  K 441
                                                    7  8
2701 AAAGAGAAGAGGAGAAGGAAAAACAAGCTGAAGAAATGGCATCAGATGATTTGTCATTAA 2760
 442   R  E  E  E  K  E  K  Q  A  E  E  M  A  S  D  D  L  S  L  I 461

2761 TTCGGAAGAACAGAATGGCTCTCTTTCAACAATTGACATGTGTGCTTCCTATCCTGGATA 2820
 462   R  K  N  R  M  A  L  F  Q  Q  L  T  C  V  L  P  I  L  D  N 481

2821 ATCTTTTAAAGGCCAATGTAATTAATAAACAGGAACATGATATTATTAAACAAAAAACAC 2880
 482   L  L  K  A  N  V  I  N  K  Q  E  H  D  I  I  K  Q  K  T  Q 501

2881 AGATACCTTTACAAGCGAGAGAACTGATTGATACCATTTTGGTTAAAGGAAATGCTGCGG 2940
 502   I  P  L  Q  A  R  E  L  I  D  T  I  L  V  K  G  N  A  A  A 521

2941 CCAACATCTTCAAAAACTGTCTAAAAGAAATTGACTCTACATTGTATAAGAACTTATTTG 3000
 522   N  I  F  K  N  C  L  K  E  I  D  S  T  L  Y  K  N  L  F  V 541
   8  9                                              9  10
3001 TGGATAAGAATATGAAGTATATCCCAACAGAAGATGTTTCAGGTCTGTCACTGGAAGAAC 3060
 542   D  K  N  M  K  Y  I  P  T  E  D  V  S  G  L  S  L  E  E  Q 561
```

Fig. 3 (cont.)

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3061 AATTGAGGAGGTTGCAAGAAGAACGAACTTGTAAAGTGTGTATGGACAAAGAAGTTTCTG 3120
 562   L  R  R  L  Q  E  E  R  T  C  K  V  C  M  D  K  E  V  S  V 581

3121 TTGTATTTATTCCTTGTGGTCATCTGGTAGTATGCCAGGAATGTGCCCCTTCTCTAAGAA 3180
 582   V  F  I  P  C  G  H  L  V  V  C  Q  E  C  A  P  S  L  R  K 601

3181 AATGCCCTATTTGCAGGGGTATAATCAAGGGTACTGTTCGTACATTTCTCTCTTAAAGAA 3240
 602   C  P  I  C  R  G  I  I  K  G  T  V  R  T  F  L  S  *       618

3241 AAATAGTCTATATTTTAACCTGCATAAAAAGGTCTTTAAAATATTGTTGAACACTTGAAG 3300
3301 CCATCTAAAGTAAAAAGGGAATTATGAGTTTTTCAATTAGTAACATTCATGTTCTAGTCT 3360
3361 GCTTTGGTACTAATAATCTTGTTTCTGAAAAGATGGTATCATATATTTAATCTTAATCTG 3420
3421 TTTATTTACAAGGGAAGATTTATGTTTGGTGAACTATATTAGTATGTATGTGTACCTAAG 3480
3481 GGAGTAGTGTCACTGCTTGTTATGCATCATTTCAGGAGTTACTGGATTTGTTGTTCTTTC 3540
3541 AGAAAGCTTTGAATACTAAATTATAGTGTAGAAAAGAACTGGAAACCAGGAACTCTGGAG 3600
3601 TTCATCAGAGTTATGGTGCCGAATTGTCTTTGGTGCTTTTCACTTGTGTTTTAAAATAAG 3660
3661 GATTTTTCTCTTATTTCTCCCCCTAGTTTGTGAGAAACATCTCAATAAAGTGCTTTAAAA 3720
3721 AGAAAAAAAAAA 3732
```

Fig. 3 (cont.)

```
SEQ. ID 9 — 1 ATTTTTTAAATTGATGCATTAACATTCTAAACATTCATCTGTTTTTAAATAGTAAAAATT 60
       61 GAACTTTGCCTTGAATATGTAATGATTCATTATAACAATTATGCATAGTCTTTAATAATC 120
      121 TGCATATTTTATGCTGCTTTCATGTTTTTCCTAATTAATGACTTCACATGTTTAATATTT 180
      181 ATAATTTTTCTGTCATAGTTTCCATATTTATATAAAATGAATACTTAAGATCAGTAATTC 240
      241 TGCTCTGTTTGTTTATATACTATTTTCCATCAAAAGACAAAATGGGACTGAGGTTGAGGC 300
      301 TCGTTGCTAAAGCACTTTCCTAAAATGCAAAAGGCCCTATGATGGATCCCTAGTACTTAT 360
      361 TTAAGTGAGAGAGAAACAGGCTGGGGGTGTAGGTCTGTTAGAGCATGTGTTTGGCATTAT 420
      421 GTGAAGCCCAAACACTAAAAAAGGAGAACAAACAAAAGCGCAGACTTTAAAACTCAAGTG 480
      481 GTTTGGTAATGTACGACTCTACTGTTTAGAATTAAAATGTGTCTTAGTTATTGTGCCATT 540
      541 ATTTTTATGTCATCACTGGATAATATATTAGTGCTTAGTATCAGAAATAGTCCTTATGCT 600
      601 TTGTGTTTTGAAGTTCCTAATGCAATGTTCTCTTTCTAGAAAAGGTGGACAAGTCCTATT 660
      661 TTCCAGAGAAGATGACTTTTAACAGTTTTGAAGGAACTAGAACTTTTGTACTTGCAGACA 720
SEQ. ID 10 — 1           M  T  F  N  S  F  E  G  T  R  T  F  V  L  A  D  T 17

      721 CCAATAAGGATGAAGAATTTGTAGAAGAGTTTAATAGATTAAAAACATTTGCTAACTTCC 780
       18   N  K  D  E  E  F  V  E  E  F  N  R  L  K  T  F  A  N  F  P 37

      781 CAAGTAGTAGTCCTGTTTCAGCATCAACATTGGCGCGAGCTGGGTTTCTTTATACCGGTG 840
       38   S  S  S  P  V  S  A  S  T  L  A  R  A  G  F  L  Y  T  G  E 57

      841 AAGGAGACACCGTGCAATGTTTCAGTTGTCATGCGGCAATAGATAGATGGCAGTATGGAG 900
       58   G  D  T  V  Q  C  F  S  C  H  A  A  I  D  R  W  Q  Y  G  D 77

      901 ACTCAGCTGTTGGAAGACACAGGAGAATATCCCCAAATTGCAGATTTATCAATGGTTTTT 960
       78   S  A  V  G  R  H  R  R  I  S  P  N  C  R  F  I  N  G  F  Y 97

      961 ATTTTGAAAATGGTGCTGCACAGTCTACAAATCCTGGTATCCAAAATGGCCAGTACAAAT 1020
       98   F  E  N  G  A  A  Q  S  T  N  P  G  I  Q  N  G  Q  Y  K  S 117

     1021 CTGAAAACTGTGTGGGAAATAGAAATCCTTTTGCCCCTGACAGGCCACCTGAGACTCATG 1080
      118   E  N  C  V  G  N  R  N  P  F  A  P  D  R  P  P  E  T  H  A 137

     1081 CTGATTATCTCTTGAGAACTGGACAGGTTGTAGATATTTCAGACACCATATACCCGAGGA 1140
      138   D  Y  L  L  R  T  G  Q  V  V  D  I  S  D  T  I  Y  P  R  N 157

     1141 ACCCTGCCATGTGTAGTGAAGAAGCCAGATTGAAGTCATTTCAGAACTGGCCGGACTATG 1200
      158   P  A  M  C  S  E  E  A  R  L  K  S  F  Q  N  W  P  D  Y  A 177

     1201 CTCATTTAACCCCCAGAGAGTTAGCTAGTGCTGGCCTCTACTACACAGGGGCTGATGATC 1260
      178   H  L  T  P  R  E  L  A  S  A  G  L  Y  Y  T  G  A  D  D  Q 197

     1261 AAGTGCAATGCTTTTGTTGTGGGGGAAAACTGAAAAATTGGGAACCCTGTGATCGTGCCT 1320
      198   V  Q  C  F  C  C  G  G  K  L  K  N  W  E  P  C  D  R  A  W 217

     1321 GGTCAGAACACAGGAGACACTTTCCCAATTGCTTTTTTGTTTTGGGCCGGAACGTTAATG 1380
      218   S  E  H  R  R  H  F  P  N  C  F  F  V  L  G  R  N  V  N  V 237

     1381 TTCGAAGTGAATCTGGTGTGAGTTCTGATAGGAATTTCCCAAATTCAACAAACTCTCCAA 1440
      238   R  S  E  S  G  V  S  S  D  R  N  F  P  N  S  T  N  S  P  R 257

     1441 GAAATCCAGCCATGGCAGAATATGAAGCACGGATCGTTACTTTTGGAACATGGACATCCT 1500
      258   N  P  A  M  A  E  Y  E  A  R  I  V  T  F  G  T  W  T  S  S 277
```

Fig. 4

```
                                                        1 2
1501 CAGTTAACAAGGAGCAGCTTGCAAGAGCTGGATTTTATGCTTTAGGTGAAGGCGATAAAG 1560
 278   V  N  K  E  Q  L  A  R  A  G  F  Y  A  L  G  E  G  D  K  V 297

1561 TGAAGTGCTTCCACTGTGGAGGAGGGCTCACGGATTGGAAGCCAAGTGAAGACCCCTGGG 1620
 298   K  C  F  H  C  G  G  G  L  T  D  W  K  P  S  E  D  P  W  D 317
                          2 3
1621 ACCAGCATGCTAAGTGCTACCCAGGGTGCAAATACCTATTGGATGAGAAGGGGCAAGAAT 1680
 318   Q  H  A  K  C  Y  P  G  C  K  Y  L  L  D  E  K  G  Q  E  Y 337
                                                   3 4
1681 ATATAAATAATATTCATTTAACCCATCCACTTGAGGAATCTTTGGGAAGAACTGCTGAAA 1740
 338   I  N  N  I  H  L  T  H  P  L  E  E  S  L  G  R  T  A  E  K  357
                             4 5
1741 AAACACCACCGCTAACTAAAAAAATCGATGATACCATCTTCCAGAATCCTATGGTGCAAG 1800
 358   T  P  P  L  T  K  K  I  D  D  T  I  F  Q  N  P  M  V  Q  E 377

1801 AAGCTATACGAATGGGATTTAGCTTCAAGGACCTTAAGAAAACAATGGAAGAAAAAATCC 1860
 378   A  I  R  M  G  F  S  F  K  D  L  K  K  T  M  E  E  K  I  Q 397

1861 AAACATCCGGGAGCAGCTATCTATCACTTGAGGTCCTGATTGCAGATCTTGTGAGTGCTC 1920
398    T  S  G  S  S  Y  L  S  L  E  V  L  I  A  D  L  V  S  A  Q 417
                                                   5 6
1921 AGAAAGATAATACGGAGGATGAGTCAAGTCAAACTTCATTGCAGAAAGACATTAGTACTG 1980
 418   K  D  N  T  E  D  E  S  S  Q  T  S  L  Q  K  D  I  S  T  E 437

1981 AAGAGCAGCTAAGGCGCCTACAAGAGGAGAAGCTTTCCAAAATCTGTATGGATAGAAATA 2040
 438   E  Q  L  R  R  L  Q  E  E  K  L  S  K  I  C  M  D  R  N  I 457

2041 TTGCTATCGTTTTTTTTCCTTGTGGACATCTGGCCACTTGTAAACAGTGTGCAGAAGCAG 2100
 458   A  I  V  F  F  P  C  G  H  L  A  T  C  K  Q  C  A  E  A  V 477

2101 TTGACAAATGTCCCATGTGCTACACCGTCATTACGTTCAACCAAAAAATTTTTATGTCTT 2160
 478   D  K  C  P  M  C  Y  T  V  I  T  F  N  Q  K  I  F  M  S  * 496

2161 AGTGGGGCACCACATGTTATGTTCTTCTTGCTCTAATTGAATGTGTAATGGGAGCGAACT 2220
2221 TTAAGTAATCCTGCATTTGCATTCCATTAGCATCCTGCTGTTTCCAAATGGAGACCAATG 2280
2281 CTAACAGCACTGTTTCCGTCTAAACATTCAATTTCTGGATCTTTCGAGTTATCAGCTGTA 2340
2341 TCATTTAGCCAGTGTTTTACTCGATTGAAACCTTAGACAGAGAAGCATTTTATAGCTTTT 2400
2401 CACATGTATATTGGTAGTACACTGACTTGATTTCTATATGTAAGTGAATTCATCACCTGC 2460
2461 ATGTTTCATGCCTTTTGCATAAGCTTAACAAATGGAGTGTTCTGTATAAGCATGGAGATG 2520
2521 TGATGGAATCTGCCCAATGACTTTAATTGGCTTATTGTAAACACGGAAAGAACTGCCCCA 2580
2581 CGCTGCTGGGAGGATAAAGATTGTTTTAGATGCTCACTTCTGTGTTTTAGGATTCTGCCC 2640
2641 ATTTACTTGGAATTTATTGGAGTTATAATGTACTTATATGATATTTCCGAA  2691
```

Fig. 4 (cont.)

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SEQ ID 11—1 TGGGAGTTCCCCGGAGCCCTGGAGGAAAGCACCGCAGGTCTGAGCAGCCCTGAGCCGGGC 60
         61 AGGGTGGGGGCAGTGGCTAAGGCCTAGCTGGGGACGATTTAAAGGTATCGCGCCACCCAG 120
        121 CCACACCCCACAGGCCAGGCGAGGGTGCCACCCCCGGAGATCAGAGGTCATTGCTGGCGT 180
                                1^2
        181 TCAGAGCCTAGGAAGTGGGCTGCGGTATCAGCCTAGCAGTAAAACCGACCAGAAGCCATG 240
        241 CACAAAACTACATCCCCAGAGAAAGACTTGTCCCTTCCCCTCCCTGTCATCTCACCATGA 300
        301 ACATGGTTCAAGACAGCGCCTTTCTAGCCAAGCTGATGAAGAGTGCTGACACCTTTGAGT 360
SEQ ID 12—1   M  V  Q  D  S  A  F  L  A  K  L  M  K  S  A  D  T  F  E  L 20

        361 TGAAGTATGACTTTTCCTGTGAGCTGTACCGATTGTCCACGTATTCAGCTTTTCCCAGGG 420
         21   K  Y  D  F  S  C  E  L  Y  R  L  S  T  Y  S  A  F  P  R  G 40

        421 GAGTTCCTGTGTCAGAAAGGAGTCTGGCTCGTGCTGGCTTTTACTACACTGGTGCCAATG 480
         41   V  P  V  S  E  R  S  L  A  R  A  G  F  Y  Y  T  G  A  N  D 60

        481 ACAAGGTCAAGTGCTTCTGCTGTGGCCTGATGCTAGACAACTGGAAACAAGGGGACAGTC 540
         61   K  V  K  C  F  C  C  G  L  M  L  D  N  W  K  Q  G  D  S  P 80

        541 CCATGGAGAAGCACAGAAAGTTGTACCCCAGCTGCAACTTTGTACAGACTTTGAATCCAG 600
         81   M  E  K  H  R  K  L  Y  P  S  C  N  F  V  Q  T  L  N  P  A 100

        601 CCAACAGTCTGGAAGCTAGTCCTCGGCCTTCTCTTCCTTCCACGGCGATGAGCACCATGC 660
        101   N  S  L  E  A  S  P  R  P  S  L  P  S  T  A  M  S  T  M  P 120

        661 CTTTGAGCTTTGCAAGTTCTGAGAATACTGGCTATTTCAGTGGCTCTTACTCGAGCTTTC 720
        121   L  S  F  A  S  S  E  N  T  G  Y  F  S  G  S  Y  S  S  F  P 140

        721 CCTCAGACCCTGTGAACTTCCGAGCAAATCAAGATTGTCCTGCTTTGAGCACAAGTCCCT 780
        141   S  D  P  V  N  F  R  A  N  Q  D  C  P  A  L  S  T  S  P  Y 160

        781 ACCACTTTGCAATGAACACAGAGAAGGCCAGATTACTCACCTATGAAACATGGCCATTGT 840
        161   H  F  A  M  N  T  E  K  A  R  L  L  T  Y  E  T  W  P  L  S 180

        841 CTTTTCTGTCACCAGCAAAGCTGGCCAAAGCAGGCTTCTACTACATAGGACCTGGAGATA 900
        181   F  L  S  P  A  K  L  A  K  A  G  F  Y  Y  I  G  P  G  D  R 200

        901 GAGTGGCCTGCTTTGCGTGCGATGGGAAACTGAGCAACTGGGAACGTAAGGATGATGCTA 960
        201   V  A  C  F  A  C  D  G  K  L  S  N  W  E  R  K  D  D  A  M 220

        961 TGTCAGAGCACCAGAGGCATTTCCCCAGCTGTCCGTTCTTAAAAGACTTGGGTCAGTCTG 1020
        221   S  E  H  Q  R  H  F  P  S  C  P  F  L  K  D  L  G  Q  S  A 240

       1021 CTTCGAGATACACTGTCTCTAACCTGAGCATGCAGACACACGCAGCCCGTATTAGAACAT 1080
        241   S  R  Y  T  V  S  N  L  S  M  Q  T  H  A  A  R  I  R  T  F 260

       1081 TCTCTAACTGGCCTTCTAGTGCACTAGTTCATTCCCAGGAACTTGCAAGTGCGGGCTTTT 1140
        261   S  N  W  P  S  S  A  L  V  H  S  Q  E  L  A  S  A  G  F  Y 280
                    2^3
       1141 ATTATACAGGACACAGTGATGATGTCAAGTGTTTTTGCTGTGATGGTGGGCTGAGGTGCT 1200
        281   Y  T  G  H  S  D  D  V  K  C  F  C  C  D  G  G  L  R  C  W 300
                                                          3^4
       1201 GGGAATCTGGAGATGACCCCTGGGTGGAACATGCCAAGTGGTTTCCAAGGTGTGAGTACT 1260
        301   E  S  G  D  D  P  W  V  E  H  A  K  W  F  P  R  C  E  Y  L 320
```

Fig. 5

```
1261 TGCTCAGAATCAAAGGCCAAGAATTTGTCAGCCAAGTTCAAGCTGGCTATCCTCATCTAC 1320
 321   L  R  I  K  G  Q  E  F  V  S  Q  V  Q  A  G  Y  P  H  L  L 340
          4 5                                                 5 6
1321 TTGAGCAGCTATTATCTACGTCAGACTCCCCAGAAGATGAGAATGCAGACGCAGCAATCG 1380
 341   E  Q  L  L  S  T  S  D  S  P  E  D  E  N  A  D  A  A  I  V 360

1381 TGCATTTTGGCCCTGGAGAAAGTTCGGAAGATGTCGTCATGATGAGCACGCCTGTGGTTA 1440
 361   H  F  G  P  G  E  S  S  E  D  V  V  M  M  S  T  P  V  V  K 380

1441 AAGCAGCCTTGGAAATGGGCTTCAGTAGGAGCCTGGTGAGACAGACGGTTCAGCGGCAGA 1500
 381   A  A  L  E  M  G  F  S  R  S  L  V  R  Q  T  V  Q  R  Q  I 400

1501 TCCTGGCCACTGGTGAGAACTACAGGACCGTCAGTGACCTCGTTATAGGCTTACTCGATG 1560
 401   L  A  T  G  E  N  Y  R  T  V  S  D  L  V  I  G  L  L  D  A 420
                                                       6 7
1561 CAGAAGACGAGATGAGAGAGGAGCAGATGGAGCAGGCGGCCGAGGAGGAGGAGTCAGATG 1620
 421   E  D  E  M  R  E  E  Q  M  E  Q  A  A  E  E  E  E  S  D  D 440

1621 ATCTAGCACTAATCCGGAAGAACAAAATGGTGCTTTTCCAACATTTGACGTGTGTGACAC 1680
 441   L  A  L  I  R  K  N  K  M  V  L  F  Q  H  L  T  C  V  T  P 460

1681 CAATGCTGTATTGCCTCCTAAGTGCAAGGGCCATCACTGAACAGGAGTGCAATGCTGTGA 1740
 461   M  L  Y  C  L  L  S  A  R  A  I  T  E  Q  E  C  N  A  V  K 480

1741 AACAGAAACCACACACCTTACAAGCAAGCACACTGATTGATACTGTGTTAGCAAAAGGAA 1800
 481   Q  K  P  H  T  L  Q  A  S  T  L  I  D  T  V  L  A  K  G  N 500

1801 ACACTGCAGCAACCTCATTCAGAAACTCCCTTCGGGAAATTGACCCTGCGTTATACAGAG 1860
 501   T  A  A  T  S  F  R  N  S  L  R  E  I  D  P  A  L  Y  R  D 520
          7 8                                                 8 9
1861 ATATATTTGTGCAACAGGACATTAGGAGTCTTCCCACAGATGACATTGCAGCTCTACCAA 1920
 521   I  F  V  Q  Q  D  I  R  S  L  P  T  D  D  I  A  A  L  P  M 540

1921 TGGAAGAACAGTTGCGGAAACTCCAGGAGGAAAGAATGTGTAAAGTGTGTATGGACCGAG 1980
 541   E  E  Q  L  R  K  L  Q  E  E  R  M  C  K  V  C  M  D  R  E 560

1981 AGGTATCCATCGTGTTCATTCCCTGTGGCCATCTGGTCGTGTGCAAAGACTGCGCTCCCT 2040
 561   V  S  I  V  F  I  P  C  G  H  L  V  V  C  K  D  C  A  P  S 580

2041 CTCTGAGGAAGTGTCCCATCTGTAGAGGGACCATCAAGGGCACAGTGCGCACATTTCTCT 2100
 581   L  R  K  C  P  I  C  R  G  T  I  K  G  T  V  R  T  F  L  S 600

2101 CCTGAACAAGACTAATGGTCCATGGCTGCAACTTCAGCCAGGAGGAAGTTCACTGTCACT 2160
      *

2161 CCCAGCTCCATTCGGAACTTGAGGCCAGCCTGGATAGCACGAGACACCGCCAAACACACA 2220
2221 AATATAAACATGAAAAACTTTTGTCTGAAGTCAAGAATGAATGAATTACTTATATAATAA 2280
2281 TTTTAATTGGTTTCCTTAAAAGTGCTATTTGTTCCCAACTCAGAAAATTGTTTTCTGTAA 2340
2341 ACATATTTACATACTACCTGCATCTAAAGTATTCATATATTCATATATTCAGATGTCATG 2400
2401 AGAGAGGGTTTTGTTCTTGTTCCTGAAAAGCAGGGATTGCCTGCACTCCTGAAATTCTCA 2460
2461 GAAAGATTTACAATGTTGGCATTTATGGTTCAGAAACTAGAATCTTCTCCCGTTGCTTTA 2520
2521 AGAACCGGGAGCACAGATGTCCATGTGTTTTATGTATAGAAATTCCTGTTATTTATTGGA 2580
2581 TGACATTTTAGGGATATGAAATTTTTATAAAGAATTTGTGAGAAAAAGTTAATAAAGCAA 2640
2641 CATAATTACCTCTTTTTTTTTAAAGAAAAAAAAAAA 2676
```

Fig. 5 (cont.)

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SEQ ID 13—1  AGTTATATAAAATACGAAGTTTTCAAAAAGAAGGCTAGTGCAACAGAAAAGCTTTGCTAA  60
          61 AACAGATTCTTAGTTATTTGAGGTAACAAAAGAAAGCCATGTCTTGAATTGATTCGTTCT 120
         121 TAATTATAACAGACTTATAGTGGAAAGGGCCTTAAACACAGGCGGACTTTATAAAATGCA 180
         181 GTCTTAGGTTTATGTGCAAAATACTGTCTGTTGACCAGATGTATTCACATGATATATACA 240
         241 GAGTCAAGGTGGTGATATAGAAGATTTAACAGTGAGGGAGTTAACAGTCTGTGCTTTAAG 300
         301 CGCAGTTCCTTTACAGTGAATACTGTAGTCTTAATAGACCTGAGCTGACTGCTGCAGTTG 360
         361 ATGTAACCCACTTTAGAGAATACTGTATGACATCTTCTCTAAGGAAAACCAGCTGCAGAC 420
         421 TTCACTCAGTTCCTTTCATTTCATAGGAAAAGGAGTAGTTCAGATGTCATGTTTAAGTCC 480
         481 TTATAAGGGAAAAGAGCCTGAATATATGCCCTAGTACCTAGGCTTCATAACTAGTAATAA 540
         541 GAAGTTAGTTATGGGTAAATAGATCTCAGGTTACCCAGAAGAGTTCATGTGACCCCCAAA 600
         601 GAGTCCTAACTAGTGTCTTGGCAAGTGAGACAGATTTGTCCTGTGAGGGTGTCAATTCAC 660
         661 CAGTCCAAGCAGAAGACAATGAATCTATCCAGTCAGGTGTCTGTGGTGGAGATCTAGTGT 720
         721 CCAAGTGGTGAGAAACTTCATCTGGAAGTTTAAGCGGTCAGAAATACTATTACTACTCAT 780
           1                                                         M  1

         781 GGACAAAACTGTCTCCCAGAGACTCGGCCAAGGTACCTTACACCAAAAACTTAAACGTAT 840
SEQ ID 14—2  D  K  T  V  S  Q  R  L  G  Q  G  T  L  H  Q  K  L  K  R  I  21

         841 AATGGAGAAGAGCACAATCTTGTCAAATTGGACAAAGGAGAGCGAAGAAAAAATGAAGTT 900
          22  M  E  K  S  T  I  L  S  N  W  T  K  E  S  E  E  K  M  K  F  41

         901 TGACTTTTCGTGTGAACTCTACCGAATGTCTACATATTCAGCTTTTCCCAGGGGAGTTCC 960
          42  D  F  S  C  E  L  Y  R  M  S  T  Y  S  A  F  P  R  G  V  P  61

         961 TGTCTCAGAGAGGAGTCTGGCTCGTGCTGGCTTTTATTATACAGGTGTGAATGACAAAGT 1020
          62  V  S  E  R  S  L  A  R  A  G  F  Y  Y  T  G  V  N  D  K  V  81

        1021 CAAGTGCTTCTGCTGTGGCCTGATGTTGGATAACTGGAAACAAGGGGACAGTCCTGTTGA 1080
          82  K  C  F  C  C  G  L  M  L  D  N  W  K  Q  G  D  S  P  V  E  101

        1081 AAAGCACAGACAGTTCTATCCCAGCTGCAGCTTTGTACAGACTCTGCTTTCAGCCAGTCT 1140
         102  K  H  R  Q  F  Y  P  S  C  S  F  V  Q  T  L  L  S  A  S  L  121

        1141 GCAGTCTCCATCTAAGAATATGTCTCCTGTGAAAAGTAGATTTGCACATTCGTCACCTCT 1200
         122  Q  S  P  S  K  N  M  S  P  V  K  S  R  F  A  H  S  S  P  L  141

        1201 GGAACGAGGTGGCATTCACTCCAACCTGTGCTCTAGCCCTCTTAATTCTAGAGCAGTGGA 1260
         142  E  R  G  G  I  H  S  N  L  C  S  S  P  L  N  S  R  A  V  E  161

        1261 AGACTTCTCATCAAGGATGGATCCCTGCAGCTATGCCATGAGTACAGAAGAGGCCAGATT 1320
         162  D  F  S  S  R  M  D  P  C  S  Y  A  M  S  T  E  E  A  R  F  181

        1321 TCTTACTTACAGTATGTGGCCTTTAAGTTTTCTGTCACCAGCAGAGCTGGCCAGAGCTGG 1380
         182  L  T  Y  S  M  W  P  L  S  F  L  S  P  A  E  L  A  R  A  G  201

        1381 CTTCTATTACATAGGGCCTGGAGACAGGGTGGCCTGTTTTGCCTGTGGTGGGAAACTGAG 1440
         202  F  Y  Y  I  G  P  G  D  R  V  A  C  F  A  C  G  G  K  L  S  221

        1441 CAACTGGGAACCAAAGGATGATGCTATGTCAGAGCACCGCAGACATTTTCCCCACTGTCC 1500
         222  N  W  E  P  K  D  D  A  M  S  E  H  R  R  H  F  P  H  C  P  241

        1501 ATTTCTGGAAAATACTTCAGAAACACAGAGGTTTAGTATATCAAATCTAAGTATGCAGAC 1560
         242  F  L  E  N  T  S  E  T  Q  R  F  S  I  S  N  L  S  M  Q  T  261
```

Fig. 6

```
1561 ACACTCTGCTCGATTGAGGACATTTCTGTACTGGCCACCTAGTGTTCCTGTTCAGCCCGA 1620
 262  H  S  A  R  L  R  T  F  L  Y  W  P  P  S  V  P  V  Q  P  E  281

1621 GCAGCTTGCAAGTGCTGGATTCTATTACGTGGATCGCAATGATGATGTCAAGTGCTTTTG 1680
 282  Q  L  A  S  A  G  F  Y  Y  V  D  R  N  D  D  V  K  C  F  C  301

1681 TTGTGATGGTGGCTTGAGATGTTGGGAACCTGGAGATGACCCCTGGATAGAACACGCCAA 1740
 302  C  D  G  G  L  R  C  W  E  P  G  D  D  P  W  I  E  H  A  K  321

1741 ATGGTTTCCAAGGTGTGAGTTCTTGATACGGATGAAGGGTCAGGAGTTTGTTGATGAGAT 1800
 322  W  F  P  R  C  E  F  L  I  R  M  K  G  Q  E  F  V  D  E  I  341

1801 TCAAGCTAGATATCCTCATCTTCTTGAGCAGCTGTTGTCCACTTCAGACACCCCAGGAGA 1860
 342  Q  A  R  Y  P  H  L  L  E  Q  L  L  S  T  S  D  T  P  G  E  361

1861 AGAAAATGCTGACCCTACAGAGACAGTGGTGCATTTTGGCCCTGGAGAAAGTTCGAAAGA 1920
 362  E  N  A  D  P  T  E  T  V  V  H  F  G  P  G  E  S  S  K  D  381

1921 TGTCGTCATGATGAGCACGCCTGTGGTTAAAGCAGCCTTGGAAATGGGCTTCAGTAGGAG 1980
 382  V  V  M  M  S  T  P  V  V  K  A  A  L  E  M  G  F  S  R  S  401

1981 CCTGGTGAGACAGACGGTTCAGCGGCAGATCCTGGCCACTGGTGAGAACTACAGGACCGT 2040
 402  L  V  R  Q  T  V  Q  R  Q  I  L  A  T  G  E  N  Y  R  T  V  421

2041 CAATGATATTGTCTCAGTACTTTTGAATGCTGAAGATGAGAGAAGAGAAGAGGAGAAGGA 2100
 422  N  D  I  V  S  V  L  L  N  A  E  D  E  R  R  E  E  E  K  E  441

2101 AAGACAGACTGAAGAGATGGCATCAGGTGACTTATCACTGATTCGGAAGAATAGAATGGC 2160
 442  R  Q  T  E  E  M  A  S  G  D  L  S  L  I  R  K  N  R  M  A  461

2161 CCTCTTTCAACAGTTGACACATGTCCTTCCTATCCTGGATAATCTTCTTGAGGCCAGTGT 2220
 462  L  F  Q  Q  L  T  H  V  L  P  I  L  D  N  L  L  E  A  S  V  481

2221 AATTACAAAACAGGAACATGATATTATTAGACAGAAAACACAGATACCCTTACAAGCAAG 2280
 482  I  T  K  Q  E  H  D  I  I  R  Q  K  T  Q  I  P  L  Q  A  R  501

2281 AGAGCTTATTGACACCGTTTTAGTCAAGGGAAATGCTGCAGCCAACATCTTCAAAAACTC 2340
 502  E  L  I  D  T  V  L  V  K  G  N  A  A  A  N  I  F  K  N  S  521

2341 TCTGAAGGAAATTGACTCCACGTTATATGAAAACTTATTTGTGGAAAAGAATATGAAGTA 2400
 522  L  K  E  I  D  S  T  L  Y  E  N  L  F  V  E  K  N  M  K  Y  541

2401 TATTCCAACAGAAGACGTTTCAGGCTTGTCATTGGAAGAGCAGTTGCGGAGATTACAAGA 2460
 542  I  P  T  E  D  V  S  G  L  S  L  E  E  Q  L  R  R  L  Q  E  561

2461 AGAACGAACTTGCAAAGTGTGTATGGACAGAGAGGTTTCTATTGTGTTCATTCCGTGTGG 2520
 562  E  R  T  C  K  V  C  M  D  R  E  V  S  I  V  F  I  P  C  G  581

2521 TCATCTAGTAGTCTGCCAGGAATGTGCCCCTTCTCTAAGGAAGTGCCCCATCTGCAGGGG 2580
 582  H  L  V  V  C  Q  E  C  A  P  S  L  R  K  C  P  I  C  R  G  601

2581 GACAATCAAGGGGACTGTGCGCACATTTCTCTCATGAGTGAAGAATGGTCTGAAAGTATT 2640
 602  T  I  K  G  T  V  R  T  F  L  S  *                          612
```

Fig. 6 (cont.)

```
2641 GTTGGACATCAGAAGCTGTCAGAACAAAGAATGAACTACTGATTTCAGCTCTTCAGCAGG 2700
2701 ACATTCTACTCTCTTTCAAGATTAGTAATCTTGCTTTATGAAGGGTAGCATTGTATATTT 2760
2761 AAGCTTAGTCTGTTGCAAGGGAAGGTCTATGCTGTTGAGCTACAGGACTGTGTCTGTTCC 2820
2821 AGAGCAGGAGTTGGGATGCTTGCTGTATGTCCTTCAGGACTTCTTGGATTTGGAATTTGT 2880
2881 GAAAGCTTTGGATTCAGGTGATGTGGAGCTCAGAAATCCTGAAACCAGTGGCTCTGGTAC 2940
2941 TCAGTAGTTAGGGTACCCTGTGCTTCTTGGTGCTTTTCCTTTCTGGAAAATAAGGATTTT 3000
3001 TCTGCTACTGGTAAATATTTTCTGTTTGTGAGAAATATATTAAAGTGTTTCTTTTAAAGG 3060
3061 CGTGCATCATTGTAGTGTGTGCAGGGATGTATGCAGGCAAAACACTGTGTATATAATAAA 3120
3121 TAAATCTTTTTAAAAAGTGTAAAAAAAAAAA 3151
```

Fig. 6 (cont.)

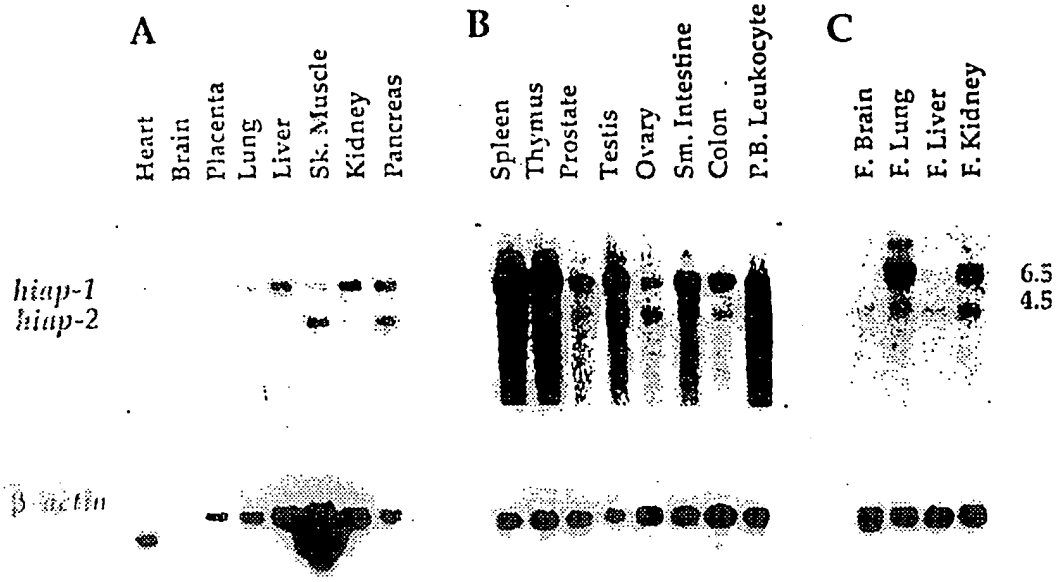

A
Heart
Brain
Placenta
Lung
Liver
Sk. Muscle
Kidney
Pancreas
B
Spleen
Thymus
Prostate
Testis
Ovary
Sm. Intestine
Colon
P.B. Leukocyte
C
F. Brain
F. Lung
F. Liver
F. Kidney
hiap-1
hiap-2
β-actin
6.5
4.5

Fig. 7

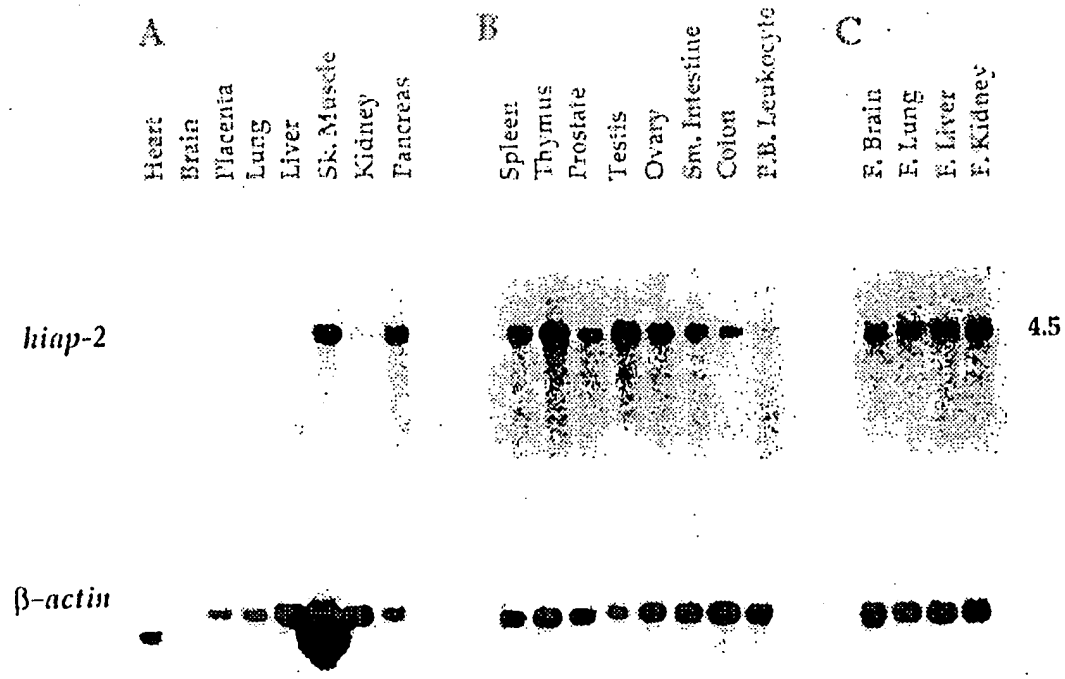

A
Heart
Brain
Placenta
Lung
Liver
Sk. Muscle
Kidney
Pancreas
B
Spleen
Thymus
Prostate
Testis
Ovary
Sm. Intestine
Colon
P.B. Leukocyte
C
F. Brain
F. Lung
F. Liver
F. Kidney
hiap-2
β-actin
4.5

Fig. 8

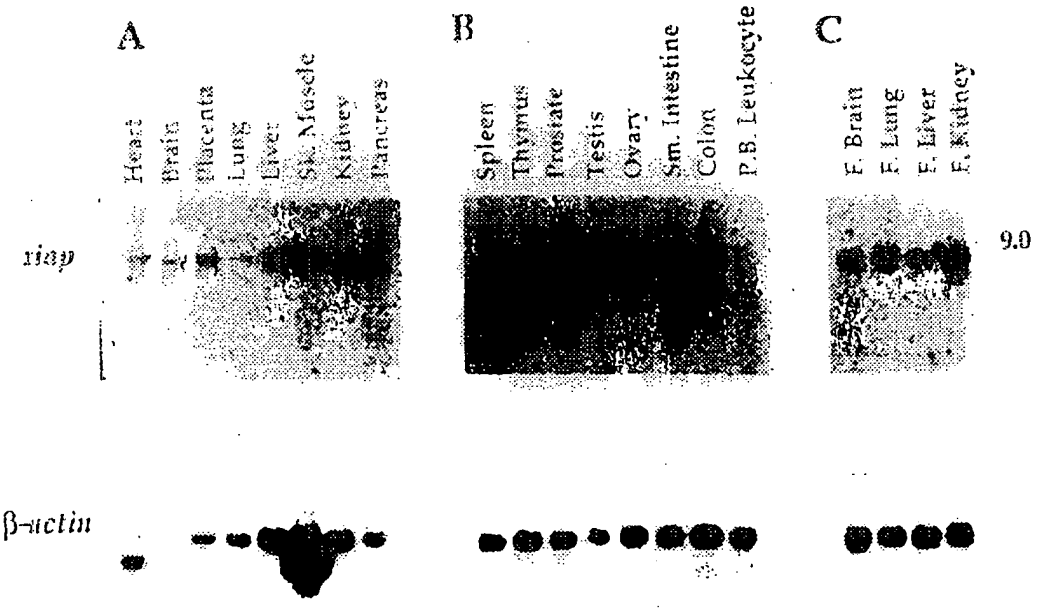

A
B
C
Heart
Brain
Placenta
Lung
Liver
Sk. Muscle
Kidney
Pancreas
Spleen
Thymus
Prostate
Testis
Ovary
Sm. Intestine
Colon
P.B. Leukocyte
F. Brain
F. Lung
F. Liver
F. Kidney
9.0
β-actin

Fig. 9

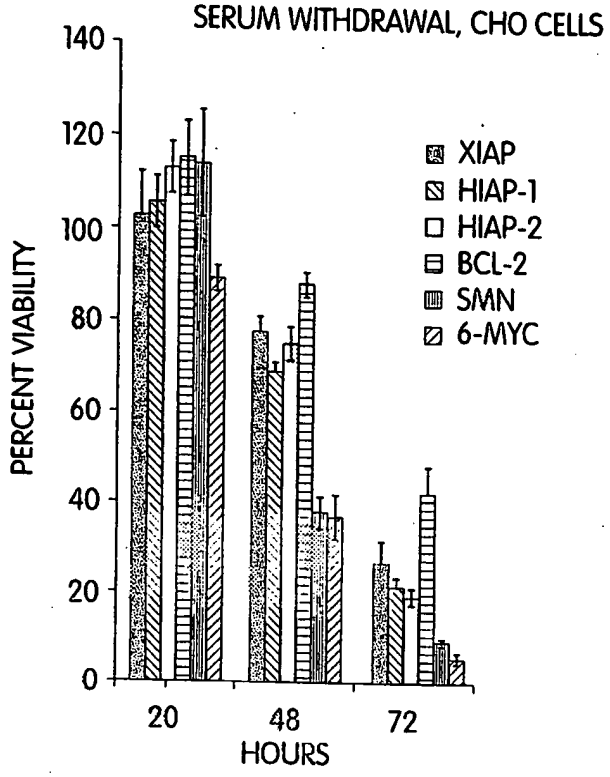



Fig. 10A

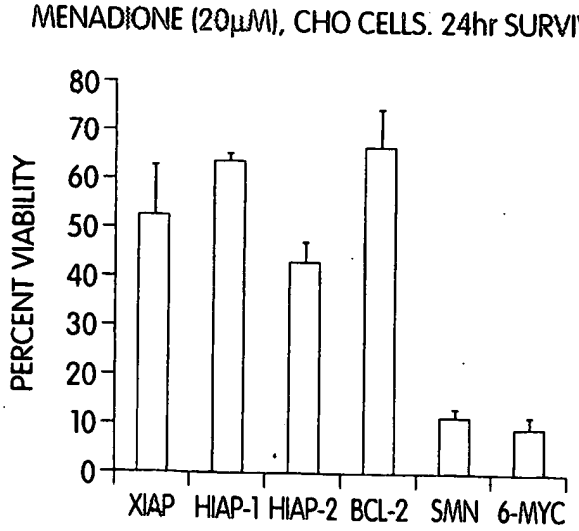



Fig. 10B

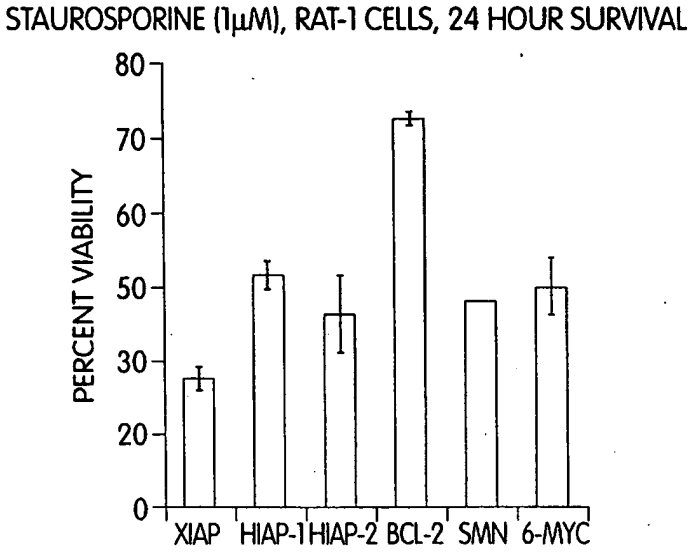



Fig. 10C

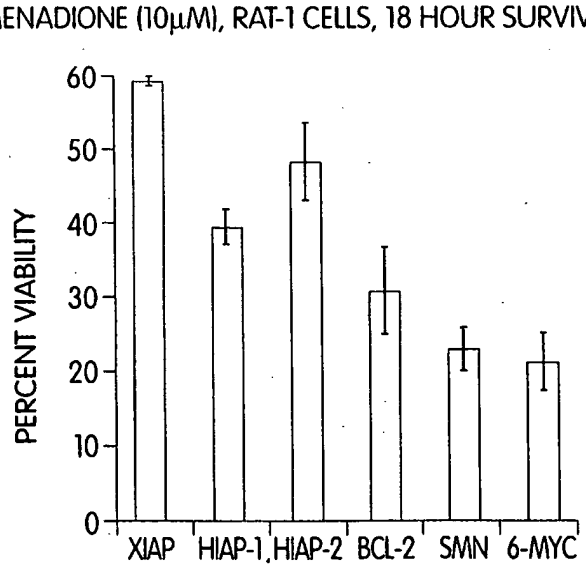



Fig. 10D

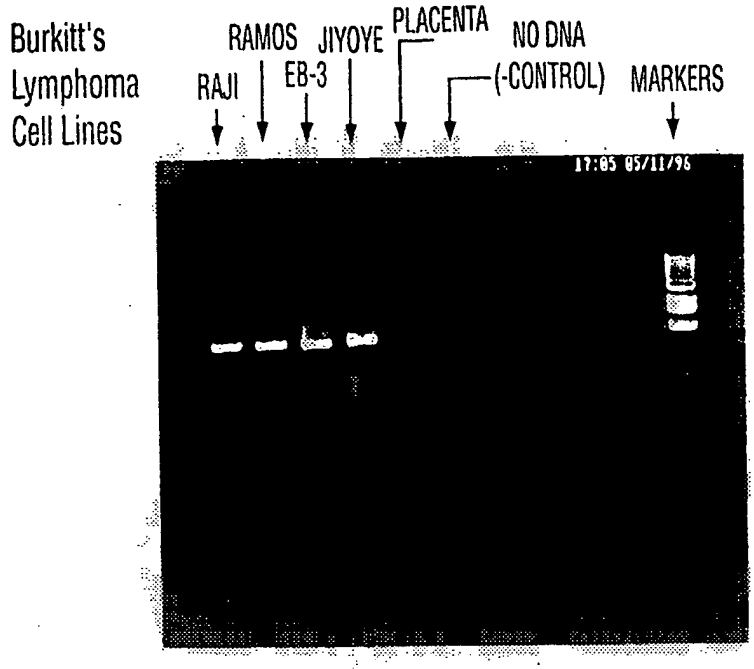

Burkitt's
Lymphoma
Cell Lines
RAJI
RAMOS
EB-3
JIYOYE
PLACENTA
NO DNA
(-CONTROL)
MARKERS
17:05 05/11/98

Fig. 11

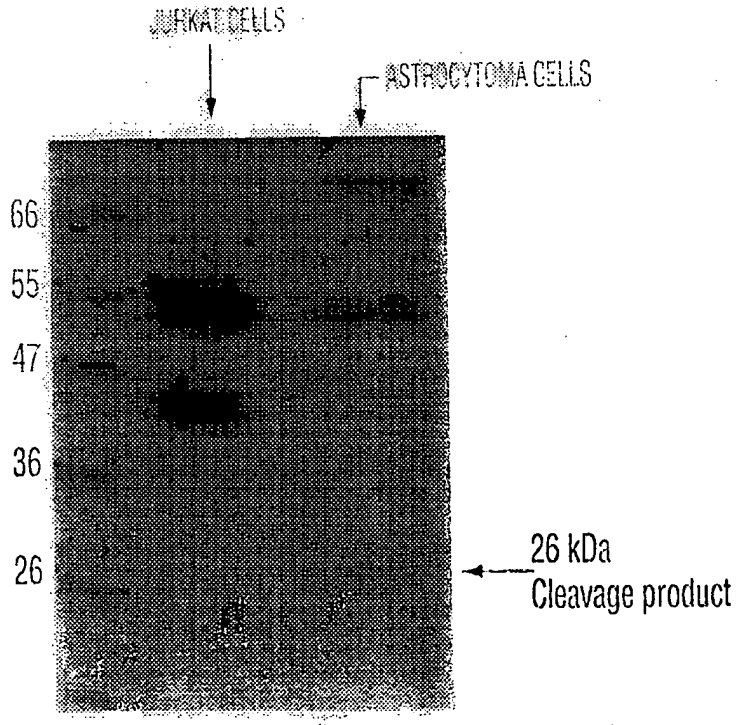

JURKAT CELLS
ASTROCYTOMA CELLS
66
55
47
36
26
26 kDa
Cleavage product

Fig. 12

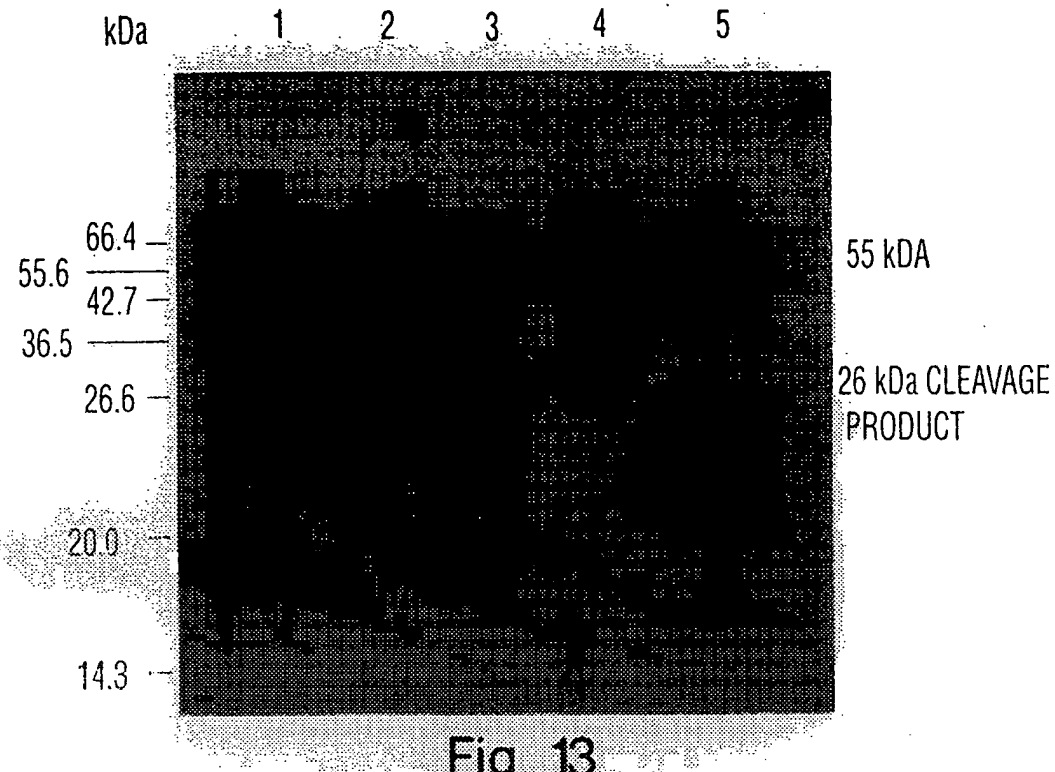

kDa
1
2
3
4
5
66.4
55.6
42.7
36.5
26.6
20.0
14.3
55 kDA
26 kDa CLEAVAGE PRODUCT

Fig. 13

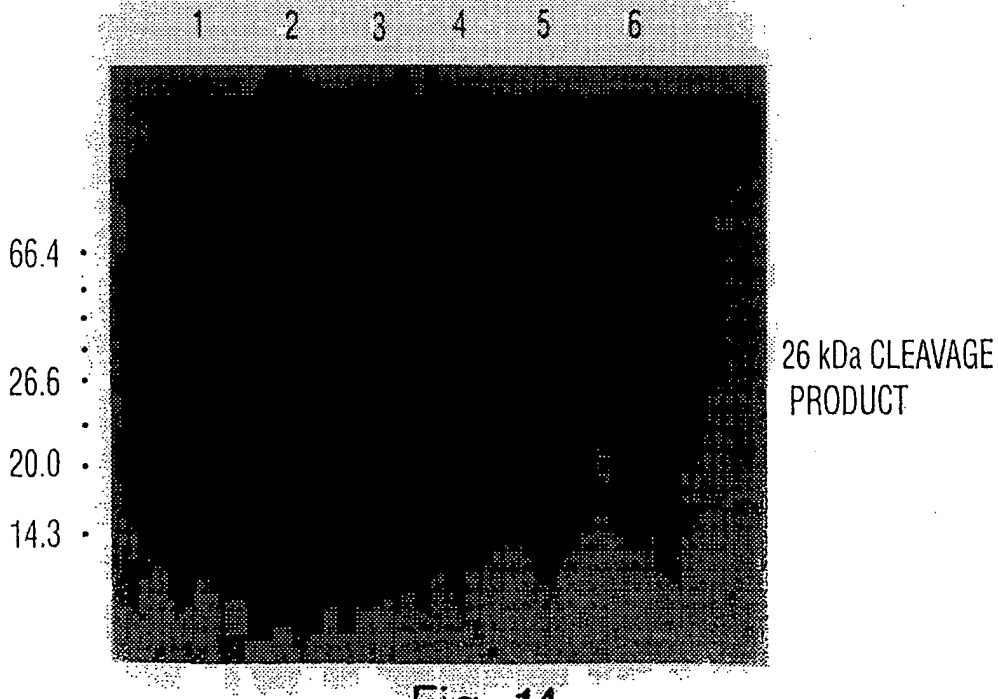

1
2
3
4
5
6
66.4
26.6
20.0
14.3
26 kDa CLEAVAGE PRODUCT

Fig. 14

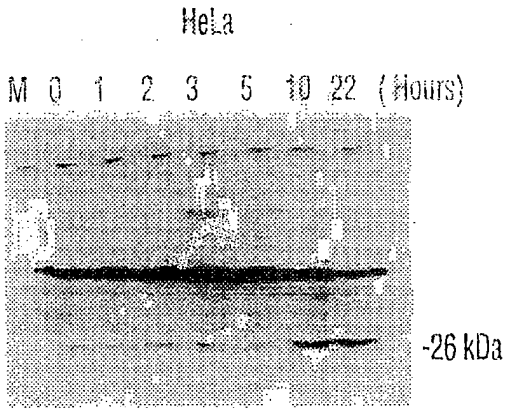



Fig. 15A

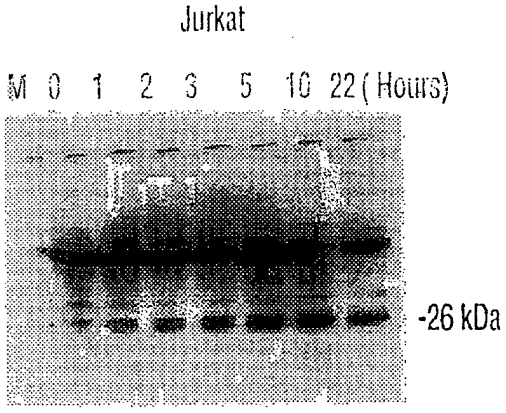



Fig. 15B

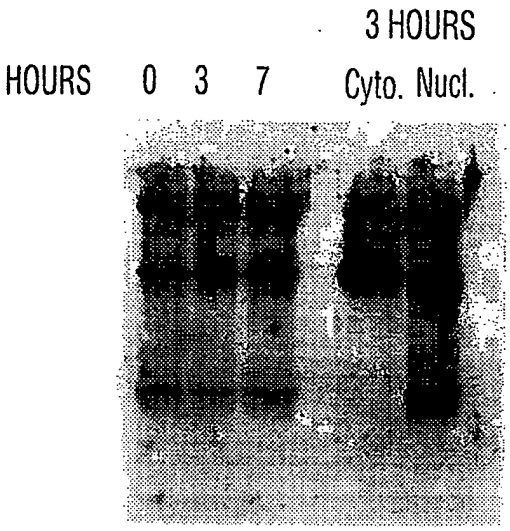



Fig. 16A

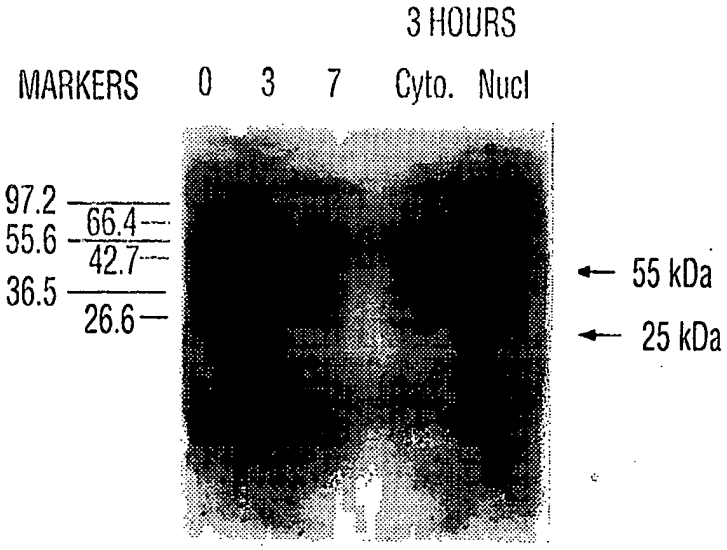



Fig. 16B

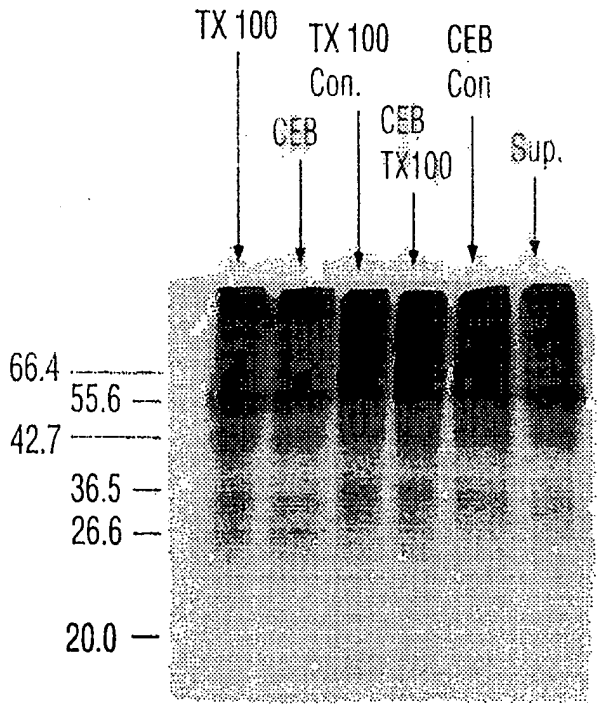

TX 100
TX 100 Con.
CEB Con
CEB
CEB TX100
Sup.
66.4
55.6
42.7
36.5
26.6
20.0

Fig. 17

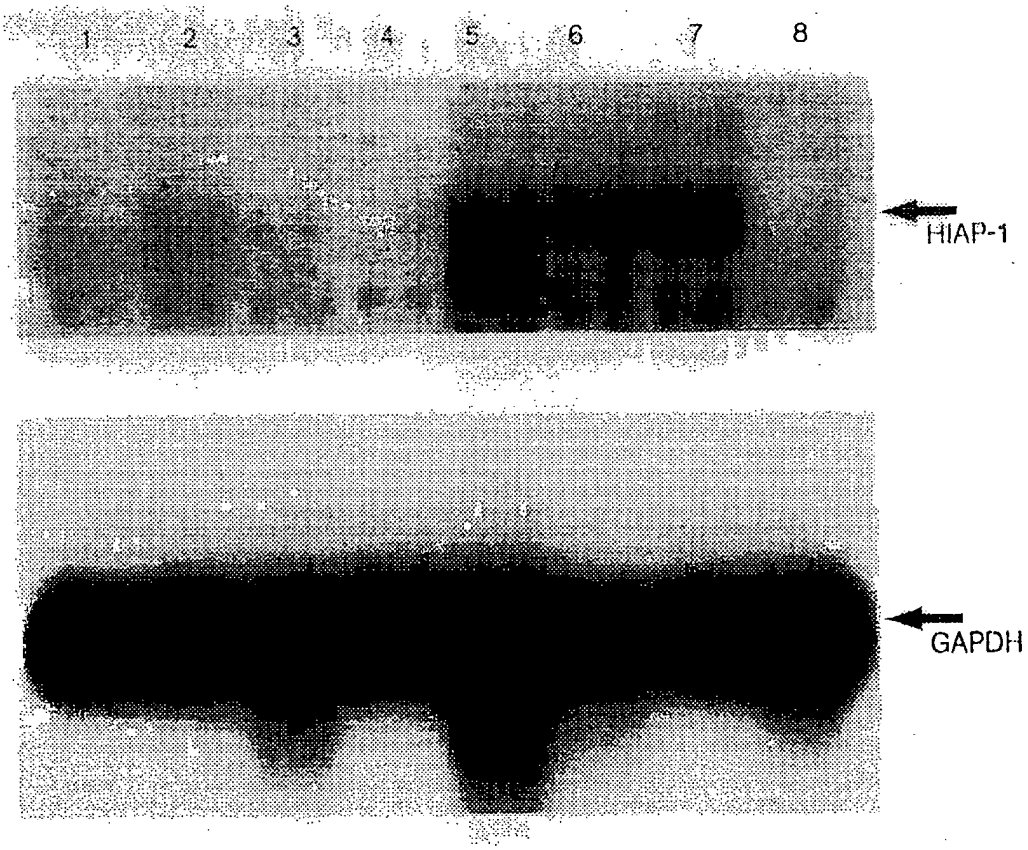



Fig. 18

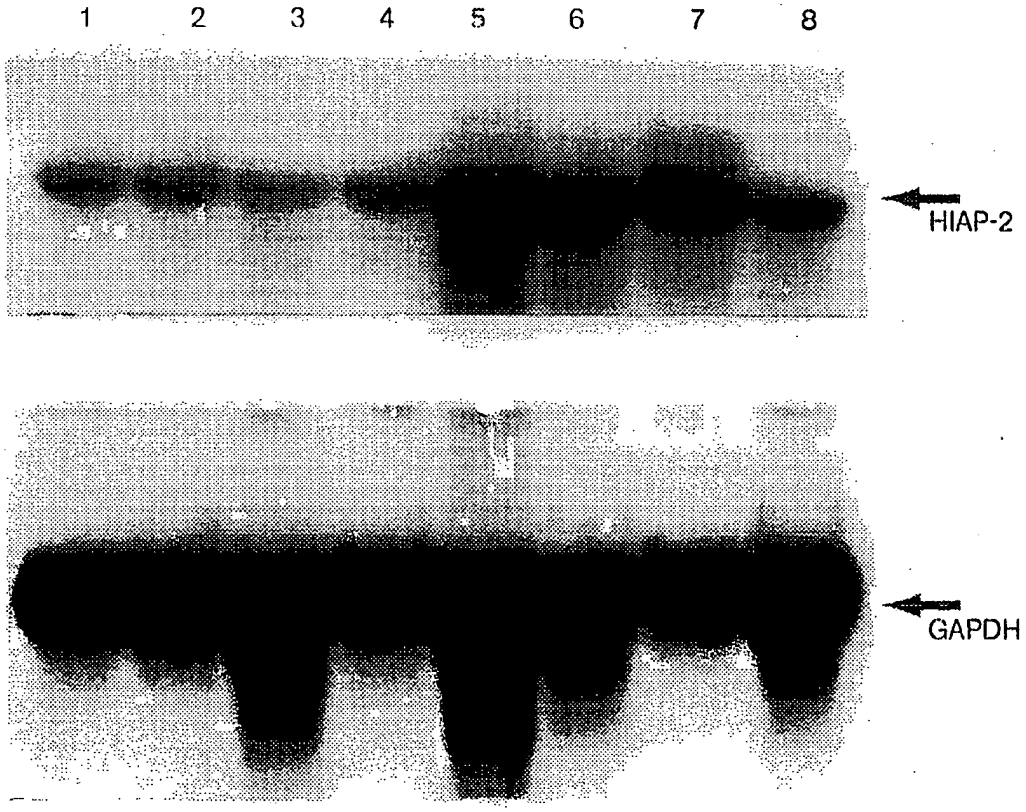



Fig. 19

INFLUENCE OF TAXOL ON DNA FRAGMENTATION IN CISPLATIN-SENSITIVE (OV2008) AND -RESISTANT (C13) HUMAN OVARIAN EPITHELIAL CANCER

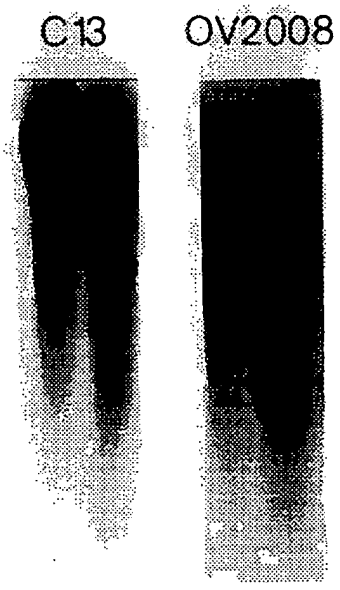



Fig. 20

SELECTIVE INFLUENCE OF CISPLATIN ON DNA FRAGMENTATION
IN SENSITIVE (OV2008) AND -RESISTANT (C13)
HUMAN OVARIAN EPITHELIAL CANCER

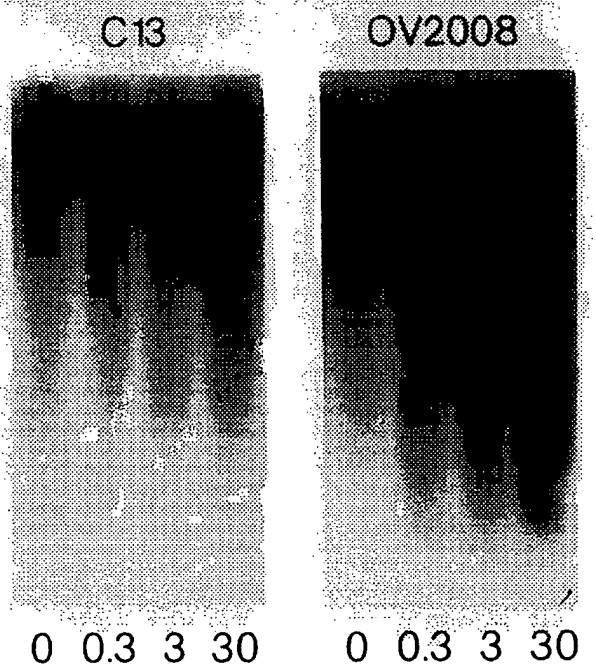



Fig. 21

EFFECTS OF TAXOL ON XIAP AND HIAP-2 PROTEIN CONTENT IN CISPLATIN-RESISTANT (C13) AND -SENSITIVE (OV2008) HUMAN OVARIAN EPITHELIAL CANCER CELLS IN VITRO

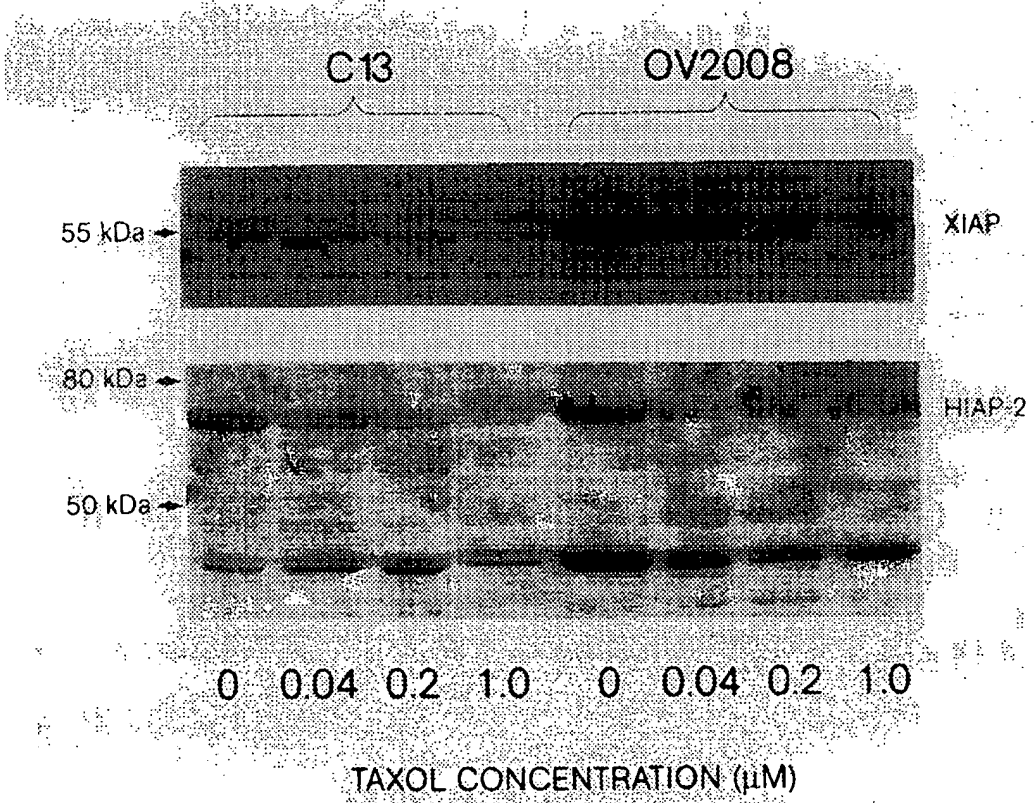



Fig. 22

INFLUENCE OF TAXOL and TGFβ ON HIAP-2 mRNA ABUNDANCE
IN CISPLATIN-SENSITIVE (OV2008) AND -RESISTANT (C13)
HUMAN EPITHELIAL CANCER CELLS IN VITRO

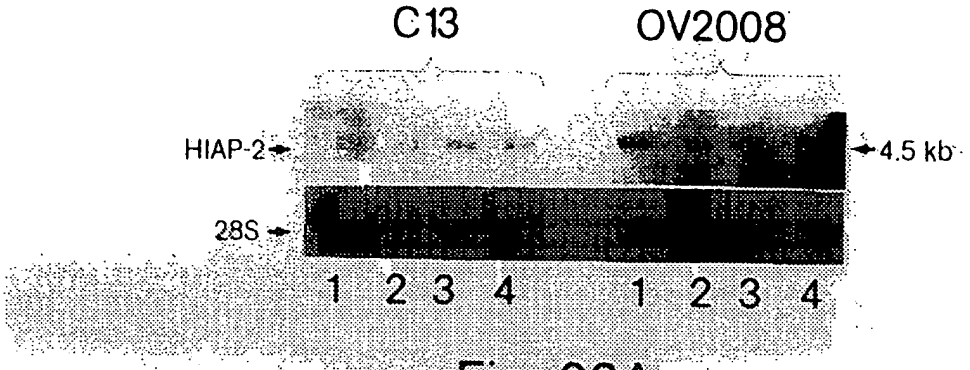
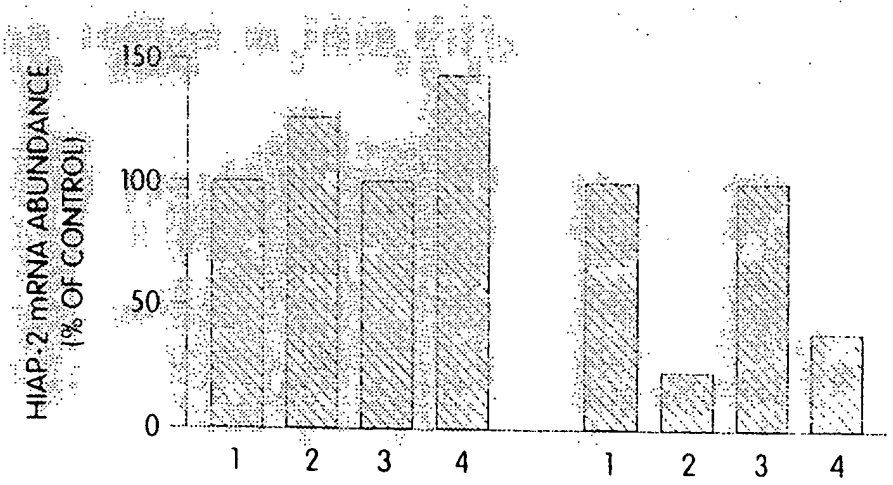



Fig. 23A




1: CONTROL
2: TAXOL (0.2 μM)
3: CONTROL
4: TGFβ (20 ng/ml))

Fig. 23B

INFLUENCE OF TGFβ ON XIAP PROTEIN EXPRESSION AND DNA FRAGMENTATION IN CISPLATIN-SENSITIVE (OV2008) AND -RESISTANT (C13) HUMAN OVARIAN EPITHELIAL CANCER CELLS IN VITRO

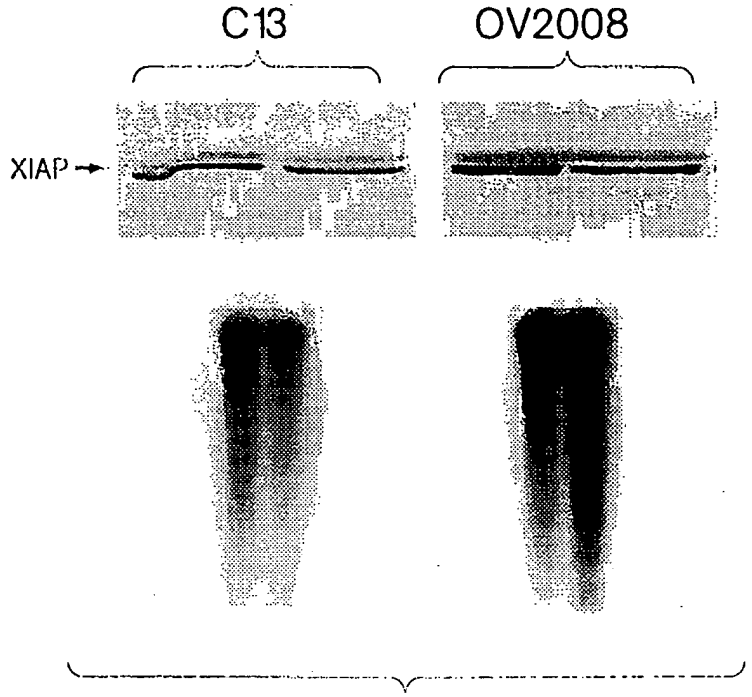



Fig. 24A

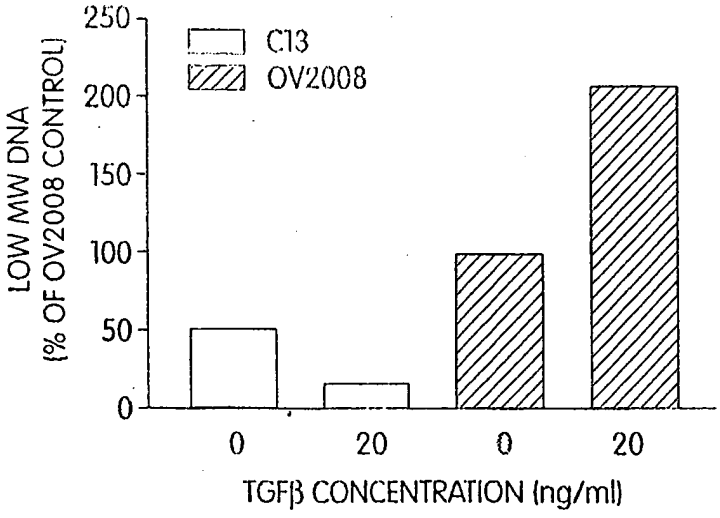



Fig. 24B

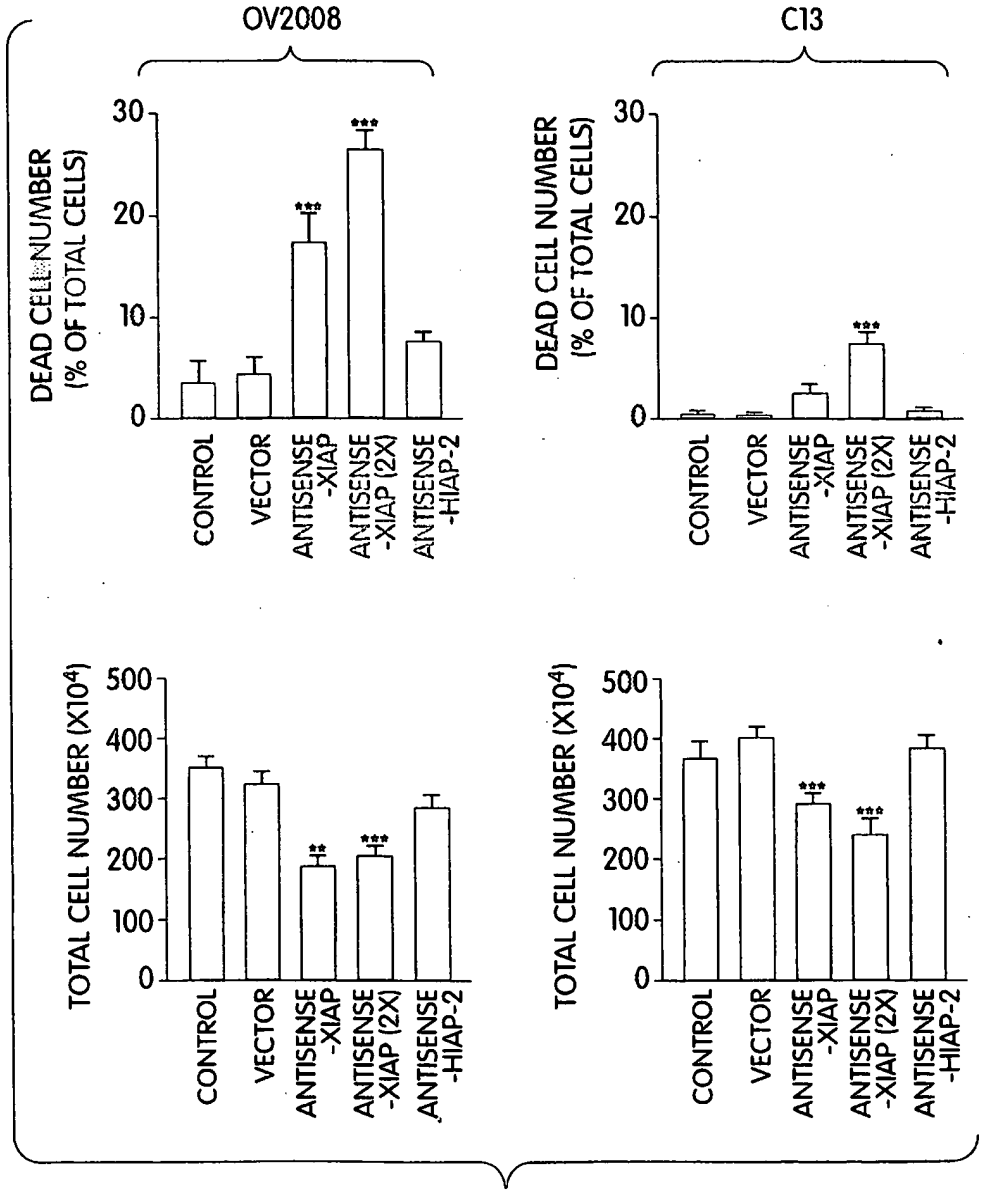

OV2008
C13
DEAD CELL NUMBER (% OF TOTAL CELLS)
30
20
10
0
***
***
***
CONTROL
VECTOR
ANTISENSE -XIAP
ANTISENSE -XIAP (2X)
ANTISENSE -HIAP-2
TOTAL CELL NUMBER (X10⁴)
500
400
300
200
100
0
**
***

Fig. 25

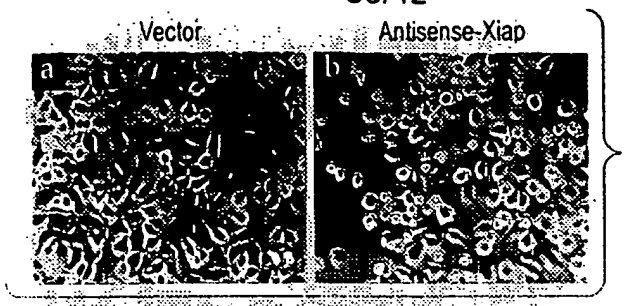



Fig. 26A

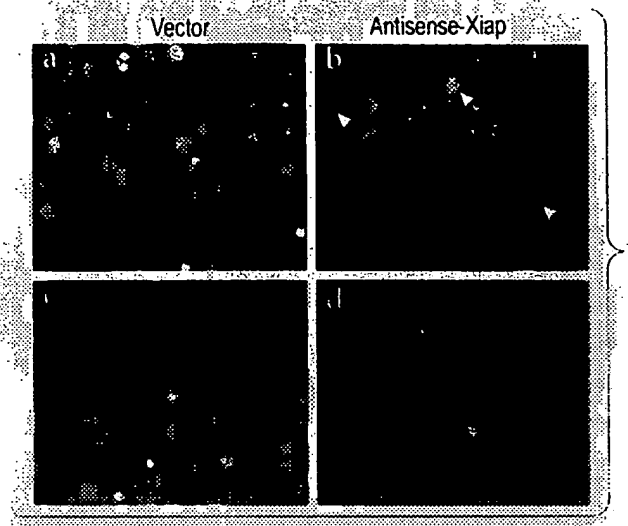



Fig. 26B

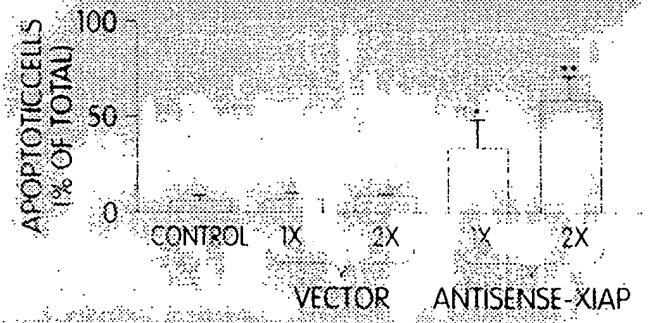



Fig. 26C

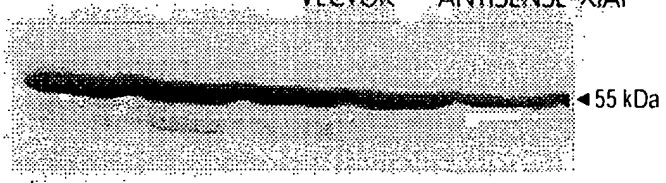



Fig. 26D

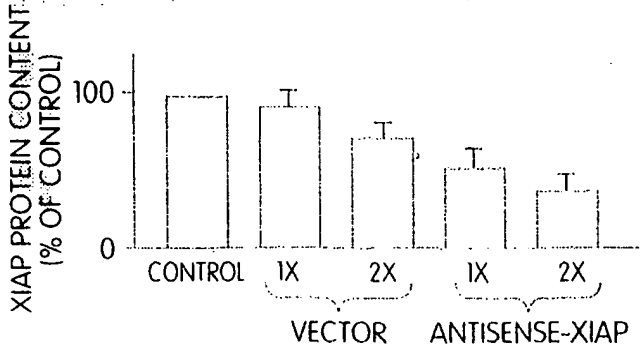



Fig. 26E

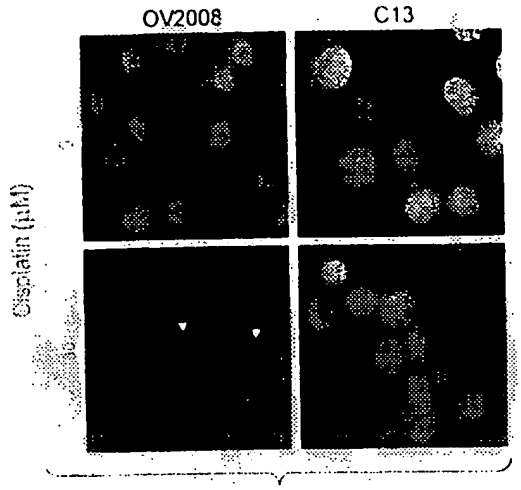



Fig. 27A

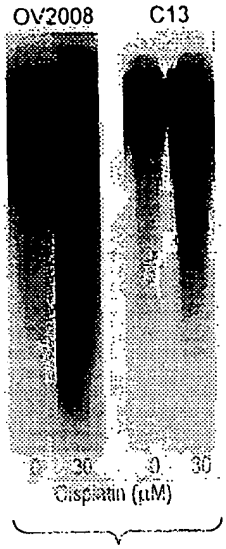



Fig. 27B

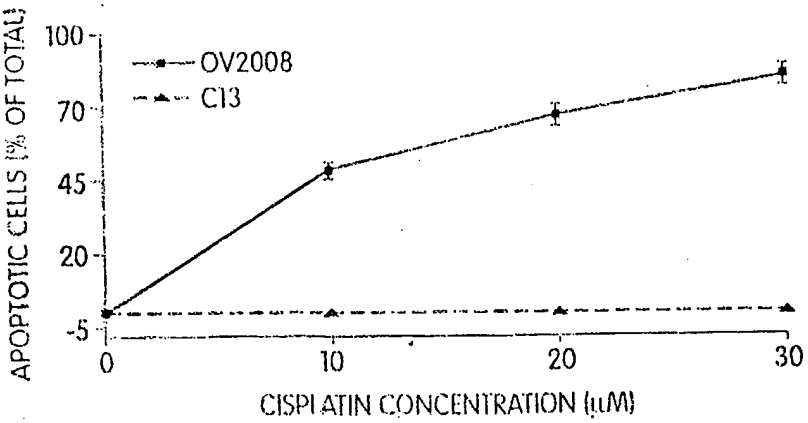



Fig. 27C

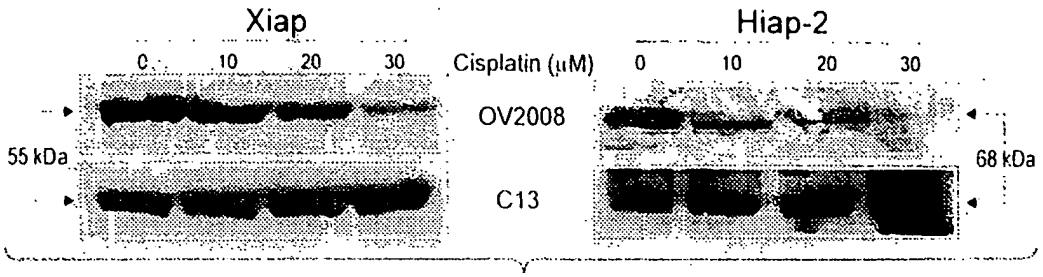



Fig. 28A

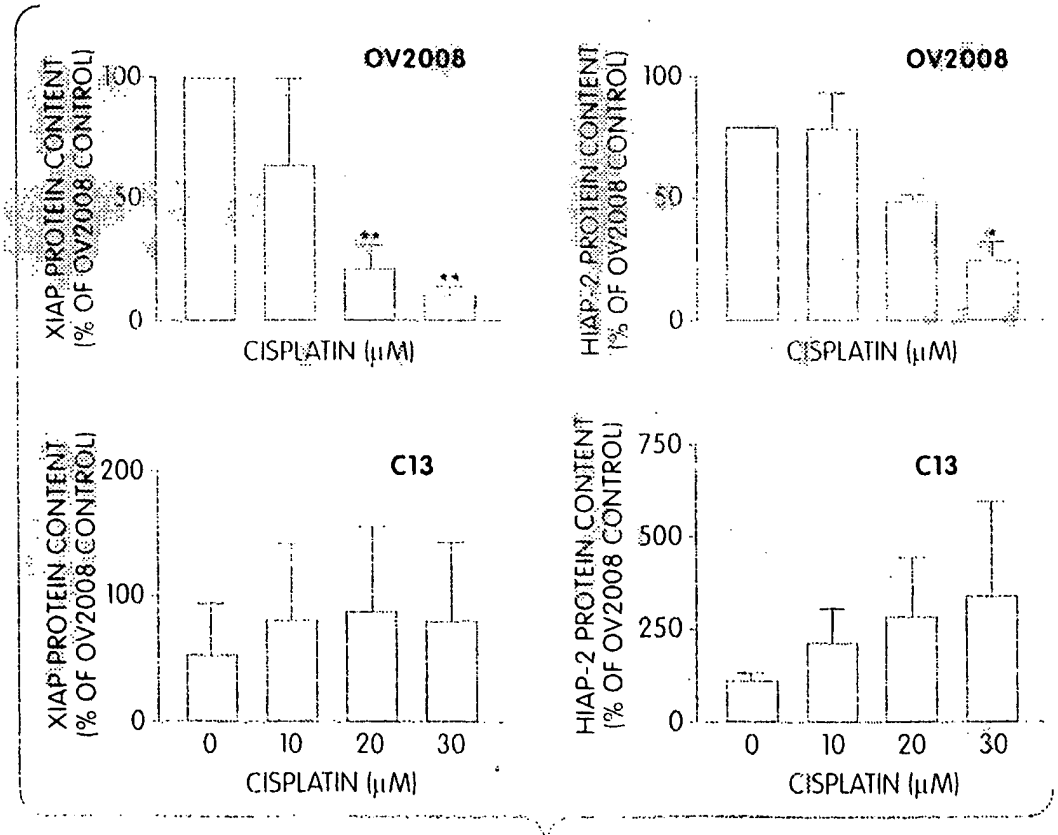



Fig. 28B

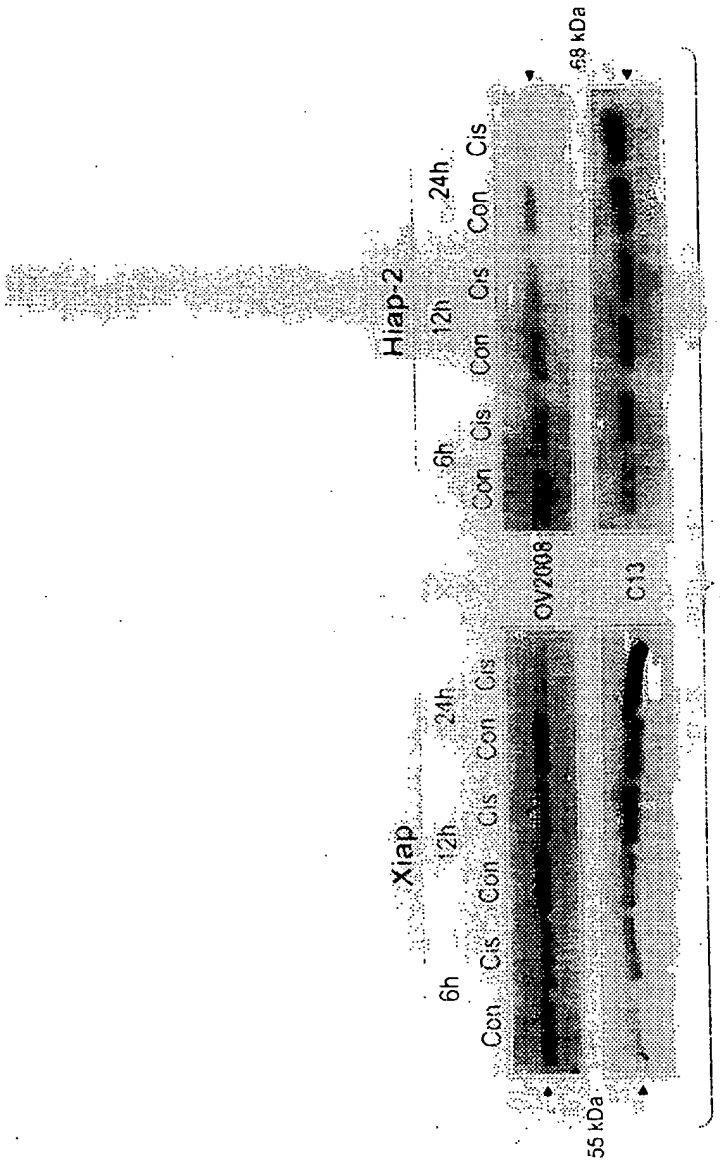



Fig. 29A

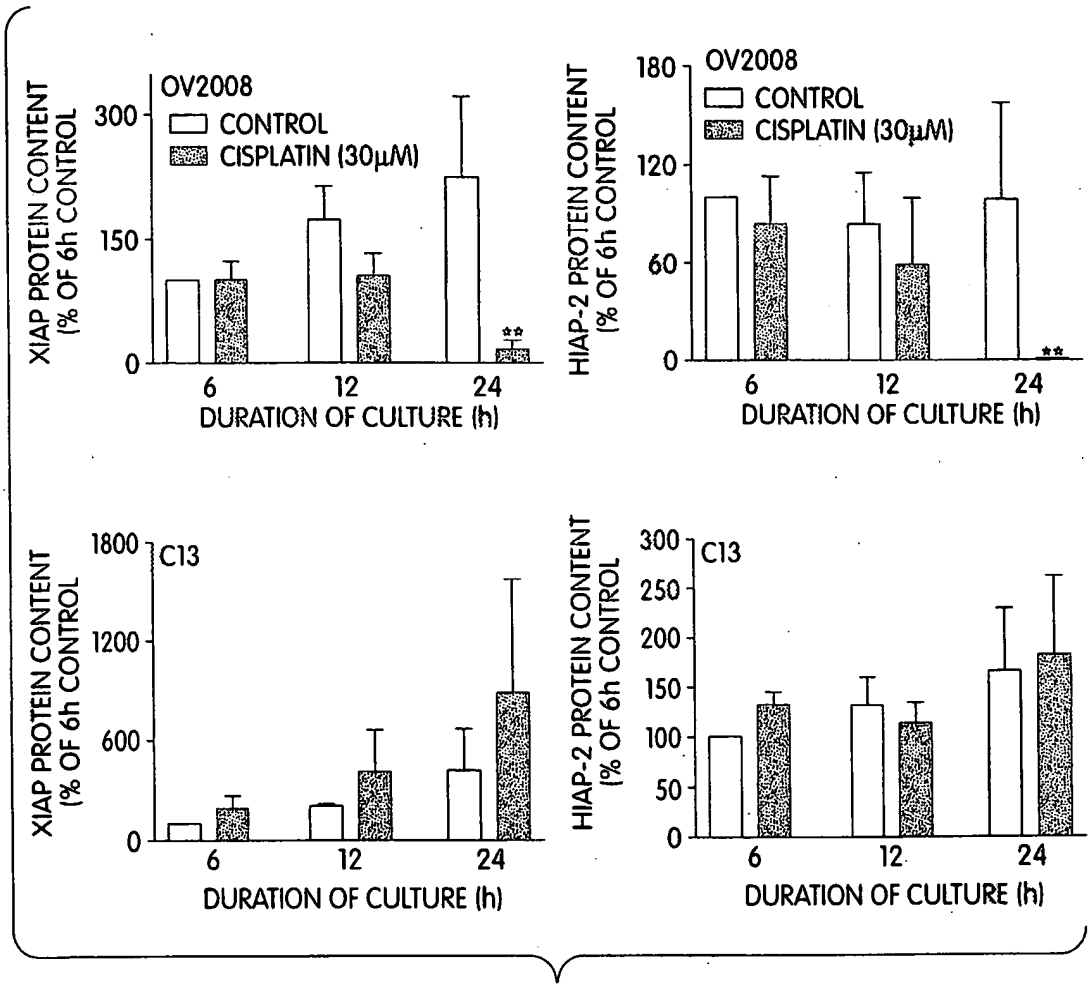

OV2008
CONTROL
CISPLATIN (30μM)
XIAP PROTEIN CONTENT (% OF 6h CONTROL
300
150
0
6
12
24
DURATION OF CULTURE (h)
**
HIAP-2 PROTEIN CONTENT (% OF 6h CONTROL
180
120
60
0
C13
1800
1200
600
250
200
100
50

Fig. 29B

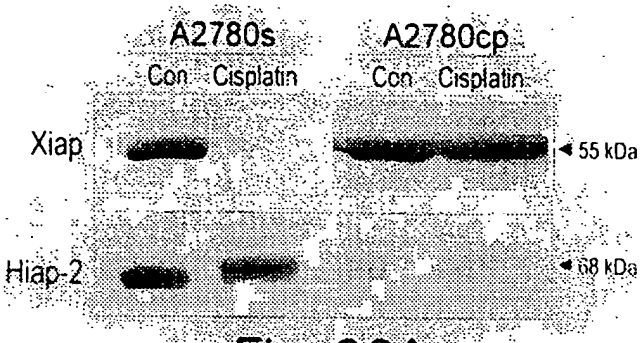



Fig. 30A

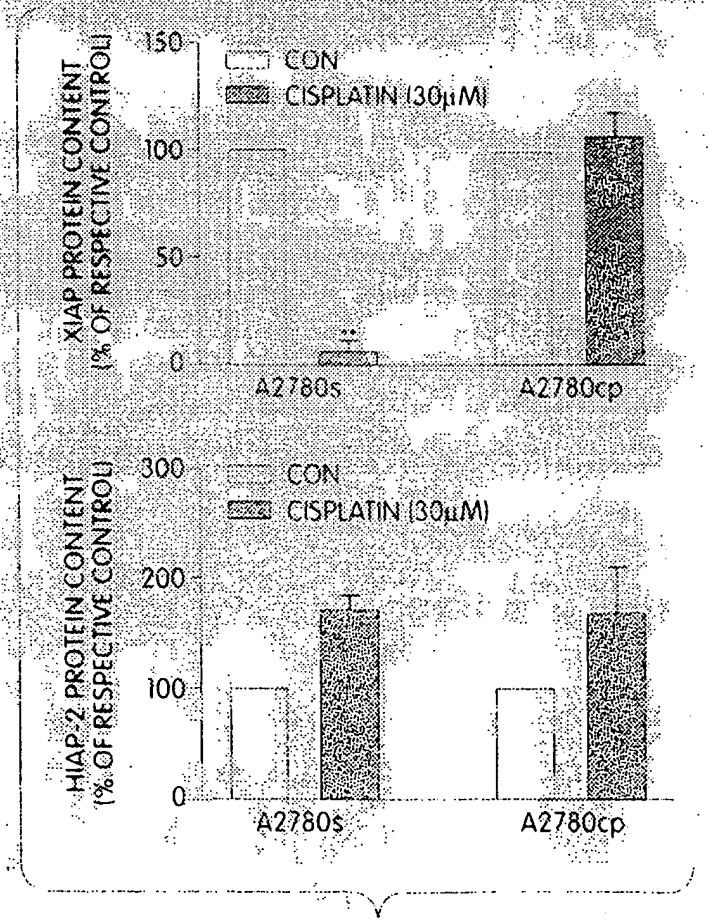



Fig. 30B

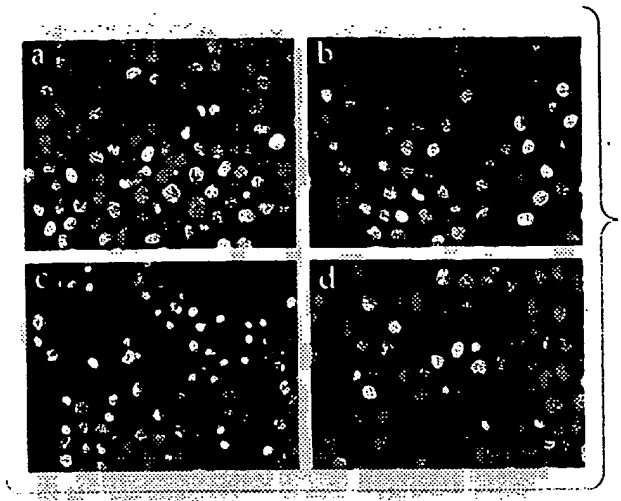



Fig. 31A

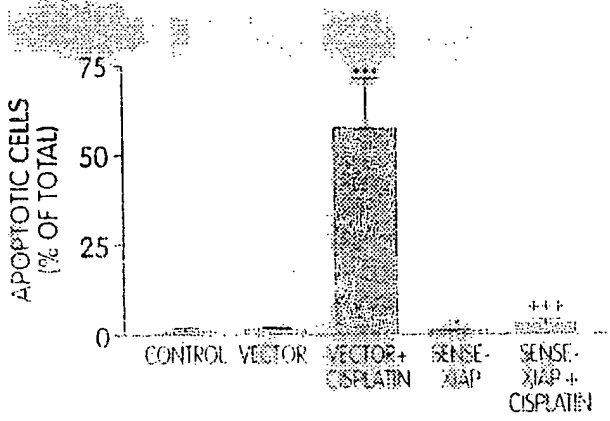



Fig. 31B

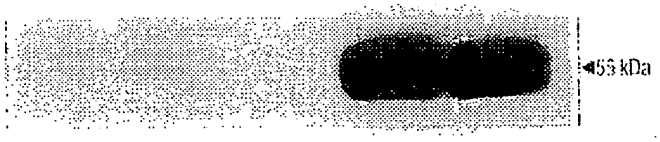



Fig. 31C

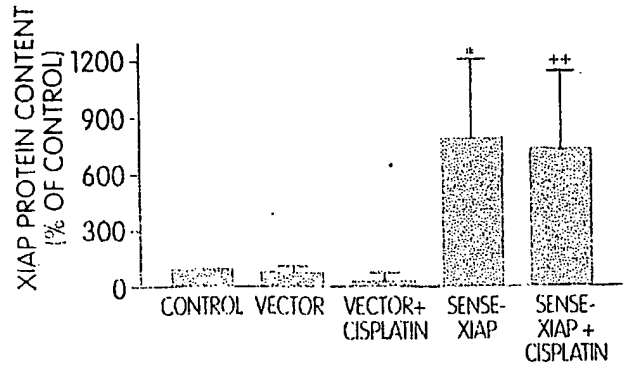



Fig. 31D

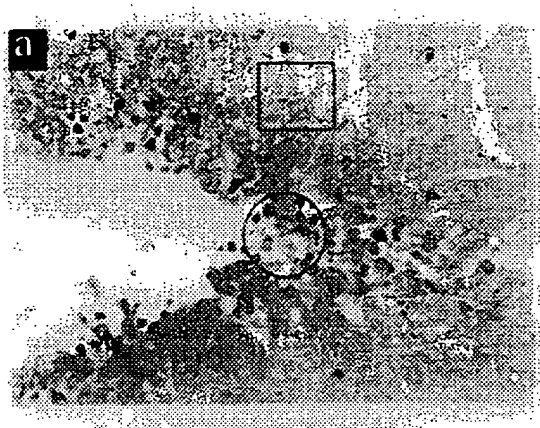



Fig. 32A

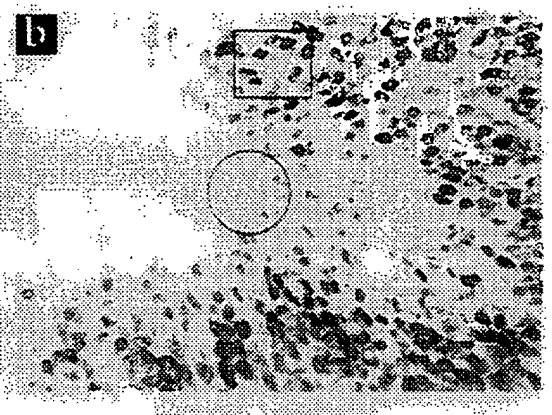



Fig. 32B

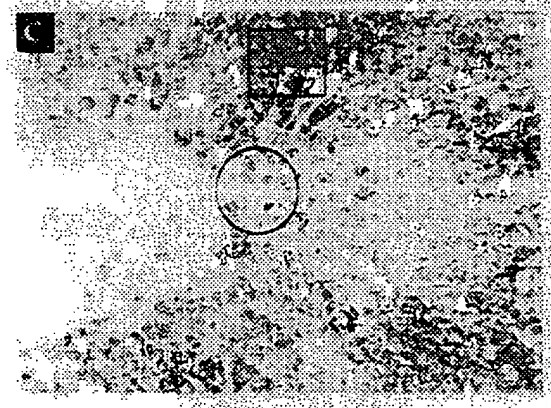



Fig. 32C

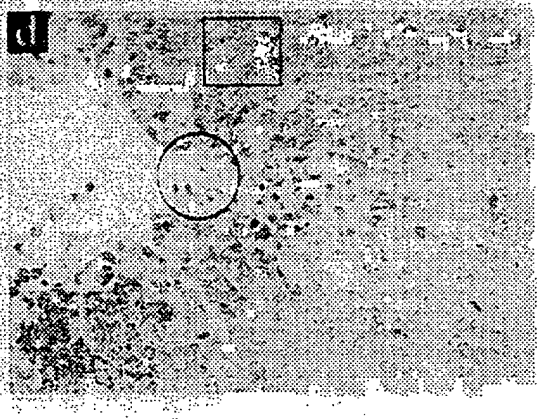



Fig. 32D

# INTERNATIONAL SEARCH REPORT

Intl. Jonal Application No
PCT/IB 98/00781

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/17 A61K31/70 A61K39/395 C12N15/11 C12Q1/68
G01N33/50 G01N33/574 A01K67/027 C12N15/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C12N C12Q G01N A01K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | P. LISTON ET AL.: "SUPPRESSION OF APOPTOSIS IN MAMMALIAN CELLS BY NAIP AND A RELATED FAMILY OF IAP GENES." NATURE, vol. 379, 25 January 1996, pages 349-353, XP002032296 LONDON GB see the whole document --- -/-- | 1-104 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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| Date of the actual completion of theinternational search | Date of mailing of the international search report |
|---|---|
| 20 August 1998 | 27/08/1998 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Ryckebosch, A |

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# INTERNATIONAL SEARCH REPORT

Int. dional Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | WO 97 06255 A (UNIVERSITY OF OTTAWA) 20 February 1997<br>cited in the application<br>see page 4, line 11 - line 28; claims 1-16,49,74,78,85-94; figures 1-4<br>see page 40, line 27 - page 43, line 21<br>see page 50, line 10 - page 52, line 8<br>see page 6, line 24 - page 7, line 9<br>see page 11, line 21 - line 29<br>see page 12, line 7 - page 13, line 9<br>see page 14, line 31 - page 15, line 9<br>see page 35, line 20 - line 23<br>see page 39, line 33 - page 40, line 2<br>--- | 1-104 |
| P,X | WO 97 26331 A (UNIVERSITY OF OTTAWA) 24 July 1997<br>cited in the application<br>see page 3, line 27 - page 4, line 8<br>see page 37, line 9 - line 11; claims 18-20,62-78<br>see page 23, paragraph 4 - page 24, line 2<br>see page 25, line 9 - page 27, line 4<br>see page 28, paragraph 4<br>see page 7, paragraph 3<br>see page 8, line 12 - line 20<br>see page 8, line 28 - page 9, line 6<br>see page 10, line 4 - line 11<br>--- | 1-104 |
| E | WO 98 22131 A (UNIVERSITY OF OTTAWA) 28 May 1998<br>cited in the application<br>see page 3, line 20 - page 4, line 3; claims 16,17,20-22,24-27,29-35<br>see page 10, line 12 - line 14<br>see page 15, line 22 - line 24<br>see page 18, line 27 - page 19, line 2<br>see page 21, line 13 - line 14<br>see page 7, line 4 - line 6<br>----- | 1-104 |

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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 98/00781

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 1-19 (as far as being related to an in vivo method) and 74-82 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. donal Application No
PCT/IB 98/00781

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9706255 A | 20-02-1997 | EP 0837939 A | 29-04-1998 |
| WO 9726331 A | 24-07-1997 | AU 1614997 A | 11-08-1997 |
| | | CA 2215793 A | 24-07-1997 |
| | | EP 0815231 A | 07-01-1998 |
| WO 9822131 A | 28-05-1998 | NONE | |

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